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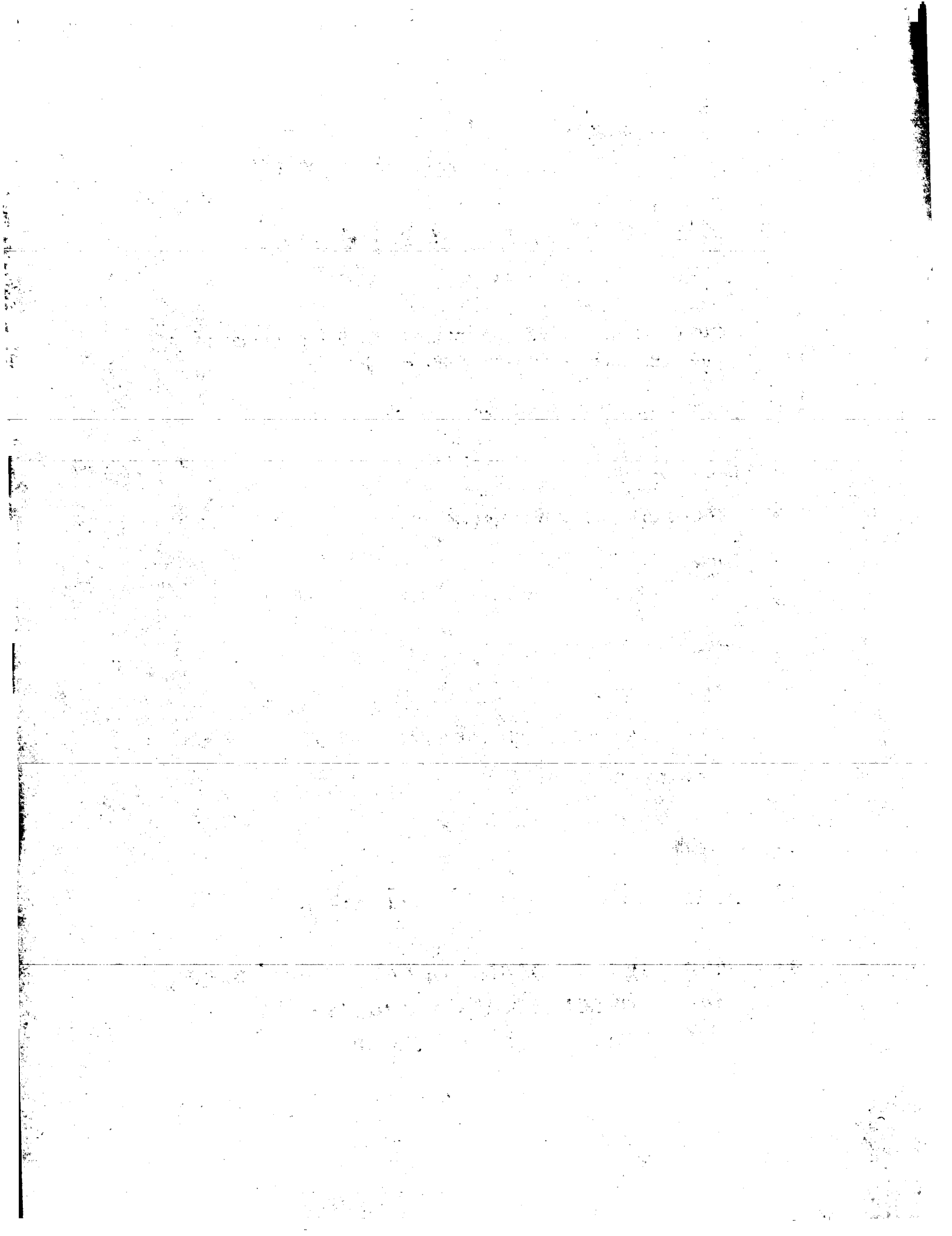
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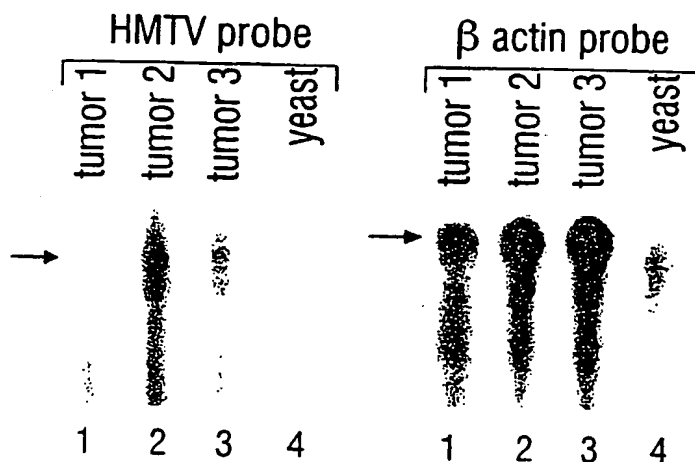
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(57) Abstract: The present invention is related to mammary tumor virus (MTV). MTV represents a group of retroviruses which possess very high homology to mouse mammary tumor virus (MMTV), a virus known to cause neoplastic mammary disease in mice. As described herein, MTV's have been identified in human, cat, and Rhesus macaque. The present invention specifically provides for recombinant nucleic acids and polypeptides derived from these MTV's as well as methods for using these biological molecules.

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## HUMAN ENDOGENOUS RETROVIRUS IN BREAST CANCER

### BACKGROUND OF THE INVENTION

The present application takes priority from Provisional U.S. patent application number 60/141,626 filed on June 30, 1999 the entire text of which is specifically incorporated herein by  
5 reference.

#### 1. FIELD OF THE INVENTION

The present invention relates to compositions and methods for the detection, prognostic evaluation, and treatment of oncogenic disorders, particularly breast cancer.

Specifically, the instant invention provides for compositions, useful for identifying and  
10 treating disorders related to newly identified endogenous retroviruses which are present in a subset of humans, cats, and nonhuman primates.

#### 2. TECHNICAL PROBLEM ADDRESSED BY THE INVENTION

##### **Mutations in known susceptibility genes do not account for all breast cancer**

Breast cancer (BC) is one of the leading causes of cancer death among women. The  
15 induction of BC is thought to involve the interplay of several factors, including the genetic, hormonal, immunological and physiological status of the host, as well as dietary habits and exposures to chemicals, radiation or infectious agents. It is now clear that variations of several genes, including BRCA-1 and BRCA-2, can result in greatly increased risks for development of BC. However, defects in known BC susceptibility genes account for only about 5% of BC, the  
20 so-called familial cases (Armstrong *et al.*, 2000; Gayther *et al.*, 1998).

As with other types of cancer, the possibility that a virus is etiologically involved in sporadically occurring BC has not been eliminated. Consequently, there has long been a need to determine which, if any viruses are causally linked to the development of BC. The identification of such a virus would likely provide invaluable aid in the following areas BC medicine:  
25 prevention, diagnosis, determination of prognosis, and treatment.

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### 3. DESCRIPTION OF RELATED ART

#### Retrovirus induction of breast cancer in mice

Mouse mammary tumor virus (MMTV), a B-type retrovirus, was discovered during studies of hereditary cancer in mice at the Jackson Laboratories in the 1930's (Bittner, 1936). As the prototype of slow-transforming retroviruses, MMTV has been definitively shown to cause BC in mice. Prior studies established that MMTV is transmitted both in the germline as endogenous proviruses and exogenously via milk. As endogenous elements MMTV proviruses follow patterns of Mendelian inheritance, as other sequences in the genome (Cohen *et al.*, Cell, 1979; Cohen and Varmus, 1979, 1980; Traina *et al.*, 1981; Traina-Dorge and Cohen, 1983; Traina-Dorge *et al.*, 1985; Varmus *et al.*, 1978). Horizontal transmission of MMTV typically occurs by infection of mouse pups by MMTV virions present in the milk of infected dams. Thus, it is possible to transmit MMTV to mice by foster feeding. 30 or more unique proviral integration sites for endogenous MMTV have been identified. However, some wild mice do not carry any endogenous MMTV proviruses (Cohen and Varmus, 1979; Cohen *et al.*, 1982). This result suggests that the many endogenous MMTV proviruses are relatively recent additions to the mouse genome. The most likely explanation is that MMTV entered the germline of certain mice (but not others) on multiple occasions after the evolutionary splits among the various species and subspecies of the genus *Mus*. Certain endogenous MMTV can be activated by hormones to form infectious virions capable of inducing mammary carcinomas after long latency periods. Most endogenous MMTV proviruses are defective and do not encode for infectious virions.

#### Roles of MMTV genes and cellular genes in oncogenesis

The MMTV Orf protein can function as a superantigen (SA). When expressed in the thymus during fetal/early development it can mediate complete or incomplete deletion of SA-reactive T-cells. SA expression is required to activate B-cells targets of MMTV in the gut-associated lymphoid tissue of nursing pups. Complete deletion of the SA responsive clones thus renders the mice resistant to MMTV infection in the gut and thereby leads to a low incidence of MMTV-induced tumors. On the other hand, in mice with only partially deleted responsive clones of lymphoid cells the SA activation stimulates expansion of the targets and spread of MMTV. As female infected animals reach puberty, estrogenic hormones drive expression of the MMTV long terminal repeat (LTR) through its hormone response element

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(HRE). This permits production and assembly of MMTV and spread of the virus to other hormonally-sensitive tissues, including the breast and ovaries. Integration of MMTV LTRs adjacent to certain cellular genes, such as the proto-oncogenes *Int*, *Wnt* and *Fgf*, can increase expression of these genes resulting in BC and other cancers.

5       The molecular genetic interactions between MMTV, the immune system of its murine host, and the breast and other hormonally-sensitive cells malignantly transformed by this retrovirus have been extensively studied. MMTV promotes mammary gland cancer in mice by insertional mutagenesis (Varmus *et al.*, 1978; Varmus, 1985). MMTV proviral LTR elements direct steroid hormone-dependent transactivation of various cellular oncogenes including *Wnt*,  
10 *Fgf* and *Int* thereby promoting clonal expansion of tumor cells (Shackleford and Varmus, 1987, Shackleford *et al.*, 1993; Jakobovits *et al.*, 1986; Nusse, 1991; Nusse *et al.*, 1985). For productive, persistent infection and completion of its replication cycle, MMTV must contain a superantigen and interact with a functional host immune system (Golovkina *et al.*, 1995; Luther and Acha-Orbea, 1996; Coffin, 1992).

#### 15   **The search for a human breast cancer virus**

      The discovery of the oncogenic MMTV has prompted many investigators to explore a retroviral etiology for BC in humans (Sarkar, 1980). Data collected over the past five decades has suggested the existence of a human homologue of MMTV. In 1971, Moore and associates reported that 60% of human milk samples from BC patients contain B-type particles  
20 indistinguishable from MMTV by electron microscopy, compared to 5% of the general population (Moore *et al.*, 1971). These investigators also reported that 39% of Parsi women of India, an inbred population with a two-fold increased incidence of BC, had B-type particles in their milk (Das *et al.*, 1972; Moore, 1971). Several studies have demonstrated that BC cells, but not cells from normal tissues, also contain reverse transcriptase (RT), an enzyme associated with  
25 all retroviruses. Numerous investigators have examined serum and breast milk for the presence of antibodies reactive with MMTV. Most of these studies were performed in the pre-AIDS era, prior to the advent of highly sensitive and specific techniques for detecting anti-retroviral antibodies made necessary for detection of HIV antibodies in donated blood.

      Despite the numerous electron microscopic, biochemical and immunological studies on  
30 human breast carcinoma tissue, milk, patients' sera, and breast carcinoma cell lines suggesting

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the existence of a human homologue of MMTV, proof that such an agent exists has remained elusive (Andersson *et al.*, 1996; Ziegler, 1997). Most authors have dismissed the importance of prior studies purporting to show evidence of a human homologue of MMTV because of the presence of numerous human endogenous retroviruses (HERVs) (Larsson *et al.*, 1994; Li *et al.*, 1996; Lower *et al.*, 1996; Meese *et al.*, 1996; Ono, 1986; Patience *et al.*, 1996; Faff *et al.*, 1992). There are about 50,000 HERVs or HERV-related sequences in the human genome, some of which have been shown to have up to 60% homology to MMTV. In this regard, it is important to note that seroreactivity to HERV-K10, to this point the HERV considered to be most closely related to MMTV, cannot account for MMTV-reactive antibodies present in the sera of breast cancer patients and the smaller number of healthy individuals (Vogetseder *et al.*, 1995). Furthermore, we believe that the presence of these MMTV-related sequences is precisely the reason that human homologues of MMTV have not previously been demonstrated conclusively by molecular techniques. The presence of these related, but distinct, sequences could have obscured the detection of more closely related sequences by prior investigators who used less sensitive techniques, such as Southern blotting.

Only recently have sequences with relatively high homology (>90%) to those of MMTV been isolated from human BC tissue (Wang *et al.*, 1995, 1998; ). Sequences 95 - 99% similar to MMTV *env* were amplified by PCR in 121 (38.5%) of 314 unselected breast cancer tumor samples. It is pertinent to note that the MMTV-like sequences were detected in only 2 (1.8%) of 107 breast specimens from reduction mammoplasties and in 0/80 samples from normal tissues or non-breast tumors. The MMTV-*env* like RNA was expressed (as determined by RT-PCR) in 66% of DNA PCR positive breast tumors (Wang *et al.*, 1998). A complete 9.9 kb provirus with 94% similarity to MMTV was detected in 2 breast tumors. FISH (fluorescence *in situ* hybridization) revealed integration at several sites in DNA derived from BC tumors, but not normal breast cells (Wang *et al.*, 1999 ACR mtg. abstracts #2933, 2944). Wang *et al.* suggested the existence of a human mammary tumor virus (HMTV) that is spread by the exogenous route of infection (horizontal transmission). Attempts by these and other investigators to amplify other regions of MMTV-related viruses, from the genomic DNA or cDNA of subjects who did not have BC, yielded HERV sequences (such as HERV-K10) with only about 60% homology to MMTV. Thus, BC tissues are the only tissues in which sequences that are highly similar to those

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of MMTV have been heretofore found. Consequently normal breast tissue and other tissues appeared to be negative for the expression of viruses with high homology to MMTV.

#### **MMTV can be transmitted horizontally or vertically**

The retrovirus replication cycle is characterized by conversion of the single-stranded  
5 RNA viral genome into doubled-stranded proviral DNA by the multiple enzymatic activities of  
the virion-associated reverse transcriptase (RT). As is the case with other retroviruses,  
integration of MMTV proviral DNA into the genome of host cells is required for expression of  
viral proteins and production of infectious progeny. In both infected mouse mammary glands as  
well as heterologous cells MMTV proviral DNA is integrated into a large number of apparently  
10 random sites. Integration of MMTV proviruses containing transcriptionally active LTR near  
some cellular genes (proto-oncogenes), such as *Int*, *Wnt*, and *Fgf* can result in over-expression of  
these genes, cellular transformation and clonal expansion of the tumor cells (Varmus, 1985;  
Shackleford *et al.*, 1993; Jakobovits *et al.*, 1986; Cohen, 1980; Breznik and Cohen, 1982). The  
long latency of MMTV-induced carcinogenesis is explained in part by the necessity for proviruses  
15 to integrate into these particular sites.

Genetic differences among viral strains of MMTV can account in part for the varying  
incidence of BC in diverse strains of mice. Mice of the C3H strain have a greater than 90%  
incidence of BC, compared to a <1% incidence of BC in BALB/c mice. BALB/c mice foster-  
nursed on C3H females have a high incidence of BC which suggested that the tumorigenic  
20 MMTV of C3H can be horizontally transmitted in the milk (Bittner, 1936). Conversely, when  
C3H mice are foster nursed on a BALB/c female the incidence of BC is significantly lower (22-  
55%), but not as low as low-incidence mouse strains. This latter observation underscores the  
importance of the horizontally-transmitted milk-borne virus in the high incidence strains, but also  
indicates the substantial differences in tumorigenic potential among endogenous MMTV  
25 proviruses. Proviruses of various mice with high and low tumor incidence could be  
distinguished by differences in solution hybridization kinetics and restriction endonuclease  
digestion patterns (Cohen *et al.* *Cell*, 1979; Cohen and Varmus, 1979, 1980; Traina-Dorge and  
Cohen, 1983; Breznik *et al.* 1984). Using restriction enzymes that differentiate between  
hypomethylated and methylated DNA, proviruses of milk-borne MMTV were also shown to be  
30 hypomethylated, whereas most endogenous proviruses contain abundant 5-methylcytosine



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(Cohen, 1980; Breznik and Cohen, 1982; Breznik *et al.*, 1984). Because hypomethylation is associated with increased gene expression, this observation could explain the importance of horizontally-transmitted milk-borne MMTV in mouse strains with a high incidence of BC. Specific hypomethylation of an endogenous MMTV provirus was associated with expression of  
5 a 1.6 kb transcript of the lactating mammary gland of the BALB/c mouse (Traina-Dorge *et al.*, 1985).

In other studies, endogenous MMTV proviruses have been shown to segregate as stable genetic units during inbreeding and that certain of these endogenous proviruses are transcriptionally active (Cohen *et al.*, *Cell*, 1979; Cohen and Varmus, 1979, 1980; Traina *et al.*,  
10 1981; Traina-Dorge and Cohen, 1983; Traina-Dorge *et al.*, 1985; Varmus *et al.*, 1978). Recombinant inbred (RI) strains of mice have been used to define MMTV provirus composition and chromosomal locations (Traina *et al.*, 1981; Traina-Dorge and Cohen, 1983). RI strains were developed by crossing mice from two highly inbred lines, randomly mating the F2 generation brothers and sisters and maintaining each as separate lines. Many genetic loci were  
15 mapped to specific chromosomes in these strains. By analyzing the segregation patterns of various MMTV proviruses with these genetic markers by restriction endonuclease analysis and Southern blotting, it was possible to determine the chromosomal locations of a number of MMTV proviruses in these strains and to establish that these provirus segregate by the rule of Mendelian inheritance. These and other analyses defined 10 distinct MMTV units (proviruses)  
20 in these animals some full length and some truncated. The ratios of inheritance for most of the MMTV proviruses were consistent with simple single-gene inheritance, though three of the MMTV units demonstrated some variance. Most noticeable was the presence of a unit contributed by one parent that was present in 23 of the 26 RI strains analyzed. Specific proviral units were identified that were transcriptionally active and associated with increased tumor  
25 production (Traina-Dorge *et al.*, 1985). Importantly, cellular genes were identified that were significantly associated with virus expression showing the involvement of host genetics in disease progression (Traina-Dorge *et al.*, 1985).

Several lines of evidence indicate that the endogenous MMTV proviruses have integrated relatively recently in the germline of various strains of mice (Cohen and Varmus, 1979; Varmus  
30 *et al.*, 1978). If MMTV evolved from elements present in a progenitor of *Mus musculus* (the laboratory mouse), it would be expected that all individual mice would have similar MMTV

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proviruses. However, both laboratory mice and wild mice trapped at several locations having quite variable numbers and distributions of germline integration sites for their MMTV proviruses (Cohen and Varmus, 1979). The most striking finding among these results were the presence of some wild caught animals that contained no endogenous MMTV proviruses. The most likely interpretation of these results is that the endogenous MMTV proviruses arose by multiple independent integrations into the DNA of germinal cells after speciation of the genus *Mus*, rather than arising from genetic elements present in the evolutionary progenitors of mice.

### SUMMARY OF THE INVENTION

The present invention provides for recombinant DNA molecules derived from one or more mammary tumor viruses which are endogenous retroviruses with homology to the sequences of MMTV. Specifically, the sequences of the instant invention have at least 99% identity with all or a portion of SEQ ID NOs: 2, 3, 4, 5, 6, 7, 8, 21, 23, 25, 27, or 29 or at least 92% identity with SEQ ID NO:10.

Additionally, the present invention provides for the RNA molecules produced by transcription of the DNA described above. Furthermore it provides for the polypeptides resulting from the in-frame translation of RNA which has been transcribed from the MTV DNA of the instant invention. According to the present invention, the referenced DNA sequences may be derived from any suitable source, such sources may include, but are not limited to, human, cat, and rhesus macaque.

The present invention also provides for a recombinant DNA plasmid (a vector) which comprises mammary tumor virus (MTV) DNA the sequences of SEQ ID NOs: 2, 3, 4, 5, 6, 7, 8, 10, 21, 23, 25, 27, or 29, or sequences which have at least 99% identity thereto. That is said DNA sequence is incorporated in the vector.

In one embodiment of the present invention the vectors further comprise a heterologous promoter operably linked to the MTV sequence (*i.e.* joined in the proper reading frame so as to be capable of producing functional MTV RNA and/or protein *in vivo* and *in vitro*). In one aspect of this embodiment of the invention the vector, which comprises the MTV DNA, is capable of episomal replication or chromosomal integration in at least one of the following cell types: bacterial cells, yeast cells, insect cells, avian cells, and mammalian cells (this list of cell types is representative and should not be considered exhaustive). In another aspect, of this embodiment

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of the invention, the heterologous promoter provides for the expression of the MTV DNA sequence in one or more cell types. Cell types considered useful as part of this aspect of the invention include, but are not limited to the following: bacterial cells, yeast cells, insect cells, avian, cells, and mammalian cells.

5 According to another embodiment of the instant invention the MTV DNA sequences described above may be used to provide a method of detecting the presence of MTV DNA or RNA in a sample (of biological origin, such as serum, or otherwise).

Another embodiment of the instant invention provides for a method of determining whether a sample contains antibodies which recognize proteins derived from the MTV DNA  
10 sequences described above (e.g. polypeptide sequences derived from transcription and translation of SEQ ID NOs: : 2, 3, 4, 5, 6, 7, 8, 10, 21, 23, 25, 27, or 29). As a corollary to this aspect, the instant invention also provides for antibodies which specifically detect one or more of the polypeptides of the instant invention.

Another embodiment of the invention provides for diagnostic kits useful for detecting  
15 DNA, RNA, or polypeptides, from a mammary tumor virus, in a biological or other type of sample.

Another embodiment of the instant invention provides for methods of attenuating or eliminating the activity of MTV in its host animal. Various aspects of this embodiment provide for pharmaceutical compositions comprising substances which disrupt the activity of the MTV  
20 reverse transcriptase, protease, or integrase enzymes. Other aspects of this embodiment provide for pharmaceutical compositions capable of eliciting an immune response in a host animal.

### BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by  
25 reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

Figure 1 shows the results of amplification of sequences related to MMTV from human breast cancer tissue. Panel A: DNA was extracted from human breast tumors (kindly provided by Michael Press, M.D., USC, Los Angeles or Derrick Beech M.D., TMC/UT Memphis) and  
30 PCR was performed using primers specific for the human MMTV *env*-related gene. PCR

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products were transferred to nitrocellulose by blotting and MMTV-related products were detected by hybridization to a 1.8 kb MMTV *env* probe. lane a: nonradioactive markers, not shown; lanes b-d, f-h, j: breast tumor DNA; lanes e and i: no DNA; lane k: water control; lane l positive control: MMTV *env* fragment cloned in pBluescript<sup>TM</sup>. Panel B: As a test for the integrity of the DNA from the clinical samples we amplified HERV-3 proviral DNA, a single copy human endogenous retrovirus using PCR conditions developed by Griffiths *et al.* (1997). Ethidium bromide detection (markers are visible in lane a). Same samples as Panel A except lane l: positive control, HERV3 *pol* fragment cloned in pBluescript<sup>TM</sup> (Stratagene). Visible bands were present in lanes c and g, but do not copy well.

Figure2 shows an example of the amplification of sequences related to MMTV from the blood of healthy controls. Panel A: DNA was extracted from whole blood of healthy control subjects and PCR was performed using primers specific for the human MMTV *env*-related gene and PCR products were detected by Southern hybridization. lane a: markers; lanes b-k: DNA from whole blood of healthy controls; lane l: water control; lane m positive control: MMTV *env* fragment in pBluescript<sup>TM</sup> (Stratagene). Panel B: PCR amplification of HERV-3. Ethidium bromide detection (markers are visible in lane a). Same samples as Panel A except lane m: positive control, HERV3 *pol* fragment in pBluescript<sup>TM</sup>.

Figure 3 shows an example of the detection of HMTV mRNA by ribonuclease protection assay. RNA was extracted from three HMTV PCR positive breast cancer tumors or from yeast cells and hybridized to either 189 base probe specific for HMTV or a 245 base probe for  $\beta$ -actin. Samples were then digested with RNase A/T1. The fragments of the labeled probes protected by hybridized RNA were visualized and analyzed following separation on a denaturing polyacrylamide gel. Two of the three tumor samples gave protected fragments of the expected size with the HMTV probe (arrow) (lanes 1-3), whereas all three tumor RNAs gave protected fragments with the  $\beta$ -actin probe (lanes (5-7). As expected neither probe was specifically protected by yeast RNA (lanes 4, 8).

## DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for isolated DNA, RNA, and polypeptides derived from mammary tumor virus (MTV) which is a virus with high sequence homology to mouse mammary tumor virus. The present invention further provides for diagnostic methods of using

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these macromolecules. Additionally the present invention provides pharmaceutical reagent compositions and diagnostic kits comprising MTV DNA, RNA, and/or polypeptide, which can be used according to the disclosed methods.

Various embodiments of the instant invention provide for MTV nucleic acid corresponding to at least one of the following: DNA sequences with at least 99% identity with one of the following:

- a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or
- b) at least 99% identity with one or more of the following: at least 340 contiguous bases of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360 of SEQ ID NO:5, at least 130 contiguous nucleotides of SEQ ID NO:6, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:7, at least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170 contiguous nucleotides of SEQ ID NO:23, at least 100 contiguous nucleotides of the sequence represented by nucleotides 337-462 of SEQ ID NO:25, at least 300 contiguous nucleotides of the sequence represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or
- c) at least 92% identity with SEQ ID NO:10..

Preferably the sequences have greater than 99% identity with greater than 200, 250, 300, 350 or 400 nucleotides SEQ ID NO's: 2, 3, 4, 5, 6, 7, 8, 21, 23, 25, 27, or 29. In another preferred embodiment the sequences have greater than 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO:10. Even more preferably these nucleic acids are derived from human, cat, or rhesus macaque MTV's. Most preferably the DNA is identical with all or part of SEQ ID NO's: 2, 3, 4, 5, 6, 7, 8, 10, 21, 23, 25, 27, or 29.

In one aspect of this embodiment of the invention the MTV DNA sequences described above are incorporated in a vector. In various related aspects the invention the MTV sequences are under the transcriptional control of an heterologous promoter. The vectors contemplated as

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being useful according to the instant invention are capable of expressing the MTV DNA sequences in at least one of the following cell types: insect cells, bacterial cells, avian cells, yeast cells, or mammalian cells. Furthermore the vectors of this aspect of the invention are also capable of episomal replication and/or chromosomal integration in at least one of the cell types listed.

In another aspect of this embodiment the DNA sequences comprise one or more detection moieties. Detection moieties contemplated as being suitable for the instant invention include, but are not limited to fluorescent dyes, and radioactive isotopes of phosphorous, sulfur, oxygen, carbon, or hydrogen.

In yet another aspect of this embodiment of the invention the isolated DNA molecule is suspended or dissolved in a diluent compatible with the invention. Suitable diluents do not interfere with the use of the DNA according to the various embodiments of the instant invention. Diluents useful according to this embodiment of the invention are well known to those skilled in the art. They include but are not limited to buffered aqueous solutions. These may be buffered with any compound compatible with the present invention. Exemplary buffering agents include phosphate buffers and buffers comprising trishydroxyaminomethane (Tris). Such buffered aqueous solutions may further comprise any other compound, such as sodium chloride, which will not interfere with the operation of the instant invention. According to this aspect of the instant invention the MTV DNA may be present in any suitable concentration. Preferably the concentration is from about 0.1 ng/ $\mu$ l to 100  $\mu$ g/ $\mu$ l.

Other embodiments of the instant invention provide for RNA transcripts and/or polypeptides encoded by the nucleic acids described above. In various aspects of this embodiment these RNA transcripts or polypeptides are encoded by any of the DNA sequences described above. These RNA transcripts and polypeptides are homologues of RNA transcripts and polypeptides encoded by the MMTV *env*, *gag*, or *pol* genes. In one aspect of this embodiment the RNA transcripts are encoded by the DNA sequences described above. Such RNA transcripts have a sequence identical to the disclosed DNA sequence except that the RNA contains uridine instead of thymidine. In another preferred aspect of this embodiment the purified polypeptide corresponds in sequence with all or part of an *env*, *gag*, or *pro*, protein product from a human, cat, or rhesus macaque mammary tumor virus. It is preferred that the peptides be purified polypeptides. In a preferred aspect of the present invention the purified

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polypeptides are comprised of all or part of the sequence resulting from the in-frame transcription and translation of any of the MTV DNA sequences described above. In an even more preferred aspect of the present invention the polypeptides correspond in sequence to the product of an in-frame translation of SEQ ID NO's: 2, 3, 4, 5, 6, 7, 8, 10, 21, 23, 25, 27, or 29.

5 Most preferably the polypeptide sequences of the instant invention correspond to all or at least 80 amino acids of SEQ ID NO's 12, 13, 14, 15, 16, 17, 18, 20, 22, 24, 26, or 28. The term "derived from MTV DNA" is meant to convey that the RNA or peptide corresponds in sequence to the RNA or peptide produced respectively by the transcription or transcription and in-frame translation of MTV DNA.

10 By "purified polypeptides" it is meant that the majority (greater than 50%) of the polypeptides in the sample are the MTV polypeptides. Preferably, the MTV polypeptides constitute greater than 70% of the polypeptide in the purified polypeptide sample. More preferably the MTV polypeptide constitutes greater than 90% of the polypeptide in the sample. Even more preferably the MTV polypeptide constitutes greater than 95% of the polypeptide in  
15 the sample. Additionally, the term "purified polypeptide" indicates that the sample does not contain substances which interfere with the operation of the instant invention. Purified polypeptides obtained in accordance with the current invention may be from any suitable source which is compatible with the instant invention.

Another embodiment of the instant invention provides for an antibody against an MTV  
20 polypeptide as described above. Antibodies according to this aspect of the invention are generated using one or more of the polypeptides of the instant invention as the antigenic agent. Such antibodies may be either monoclonal or polyclonal in nature. Preferably the antibodies are monoclonal. Once the MTV has been provided the antibodies of this aspect of the invention may be prepared according to methods well known to those skilled in the art. For example,  
25 polyclonal antibodies are commonly produced by injecting the antigenic agent (in the presence of an immune response enhancing agent such as complete Freund's adjuvant) into an animal such as a sheep, bovine, equine, goat, or rabbit.

Monoclonal antibodies according to this aspect of the present invention can be prepared by hybridoma fusion techniques or by techniques that utilize Epstein Barr Virus (EBV) -  
30 immortalization technologies (to produce human mAbs), such as are well known by those of skill in the art, modified as described herein. In the method of the invention, these techniques involve

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the injection of an immunogen, in this case, purified MTV polypeptide so as to elicit a desired immune response in that animal (i.e., production of antibodies). The experimental animal, (e.g., a mouse) is given repeated injections (boosts) of the same immortalized cell line. In a final step, the animal is given an injection of primary cells of the chosen cell type.

5 In the illustrative example herein for the production of agonist monoclonal antibodies to megakaryocytic cells, a CMK cell preparation and a CMS cell preparation were used as the first immunogens; however, other immortalized megakaryocytic cells, such as Mo7e or DAMI cells, could have been used. Other monoclonal antibodies analogous to the agonist antibody of the invention BAH-1, which specifically recognizes the C-Mpl receptor, can be generated using  
10 membrane bound c-Mpl receptor protein as the immunogen. To generate agonist monoclonal antibodies against other cell types, other cells of hemopoietic lineage are chosen, e.g., stem cells, B cells or T cells. In the first immunization step stem cells can be represented, e.g., by the immortalized cell line CTS; B cells by the immortalized cell lines ARH-77, SB or Nal-6; and T cells by the immortalized cell lines Jurkat or H9.

15 After a sufficient time, the animal is sacrificed and somatic antibody-producing cells may be derived from the lymph nodes, spleens and peripheral blood of primed animals. Spleen cells are preferred. Mouse lymphocytes give a higher percentage of stable fusions with the mouse myelomas described below. The use of rat, rabbit, frog, sheep and other mammalian somatic cells is also possible. The spleen cell chromosomes encoding desired immunoglobulins are  
20 immortalized by fusing the spleen cells with myeloma cells, generally in the presence of a fusing agent such as polyethylene glycol (PEG). Any of a number of myeloma cell lines may be used as a fusion partner according to standard techniques; for example, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110-2209.

25 The resulting cells, which include the desired hybridomas, are then grown in a selective medium, such as HAT medium, in which unfused parental myeloma or lymphocyte cells eventually die. Only the hybridoma cells survive and can be grown under limiting dilution conditions to obtain isolated clones. The supernatants of the hybridomas are screened for the presence of antibody of the desired specificity, e.g., by immunoassay techniques such as those  
30 described herein, using the antigen that has been used for immunization. Positive clones can then be subcloned under limiting dilution conditions and the monoclonal antibody produced can be



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isolated. Various conventional methods exist for isolation and purification of the monoclonal antibodies so as to free them from other proteins and other contaminants. Commonly used methods for purifying monoclonal antibodies include ammonium sulfate precipitation, ion exchange chromatography, and affinity chromatography. Hybridomas produced according to these methods can be propagated in vitro or in vivo (in ascites fluid) using techniques known in the art (see, generally, Harlow et al., Antibodies. A Laboratory Manual, Cold Spring Harbor Laboratory, pp. 1-726, 1988).

Other embodiments of the instant invention provide methods for detecting DNA, RNA, and/or proteins from MTV in biological and/or other types of samples.

One aspect of this embodiment of the invention provides a method for the detection of MTV DNA in a sample which comprises the following steps:

- i) obtaining an sample suspected of containing one or more of the MTV DNA sequences described above;
- ii) carrying out a polymerase chain reaction (PCR) to amplify a DNA sequence as defined in step i); and,
- iii) determining the sequence of, or otherwise characterizing, the amplicons (the PCR amplified DNA) produced in step ii) to determine whether or not the DNA sequence as defined in step i) is present in the sample.

Another aspect of this embodiment provides a method for the detection of MTV RNA in a sample which comprises the following steps:

- i) obtaining an sample suspected of containing RNA which encoded by one or more of the MTV DNA sequences described above;
- iii) carrying out an RNase protection assay (RPA); and,
- iii) analyzing the RPA results to determine whether RNA as defined in step i) is present in the sample and optionally quantitating said RNA.

It will be recognized by those of ordinary skill that the selection of the parameters necessary for optimizing the steps of these methods are well within the abilities of the ordinarily skilled artisan. These parameters, for example PCR conditions (e.g. selection of annealing temperature, extension times, and primers) and DNA sequencing method, are routinely determined in labs where such molecular biological techniques are employed.

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Another aspect of this embodiment of the invention provides for a method for analyzing a sample in order to determine whether the sample contains antibodies which recognize MTV polypeptides. This method comprises the steps of:

- i) obtaining a sample suspected of containing antibodies specific for mammary tumor viral antibodies;
- ii) obtaining at least one purified MTV polypeptide;
- iii) performing western immunoblot analysis using the sample of step i) and the polypeptide of step ii); and,
- iv) analyzing the results of step iii) to determine whether or not antibodies which specifically interact with the peptide of step ii) are present in the sample.

Another aspect of this embodiment of the invention provides for a method for analyzing a cell culture or a tissue sample by one or more immunohistological methods in order to determine whether the sample contains MTV proteins. This method comprises the steps of:

- i) obtaining a sample suspected of containing mammary tumor viral proteins;
- ii) preparing the sample of step i) for immunochemical analysis;
- iii) incubating the sample of step ii) with one or a combination of two or more monoclonal or polyclonal antibodies specific for MTV polypeptides encoded by one or more of the MTV DNA sequences described above, wherein the said antibodies optionally have a moiety which allows for their specific detection;
- iv) washing the samples to remove antibody which is not specifically bound;
- v) processing the samples as appropriate for the selected detection method; and,
- vi) analyzing the results of step v.) to determine whether or not MTV proteins are present in the sample.

It is envisioned that the immunochemical analysis may include, but is not limited to analysis by western blotting or enzyme-linked immunosorbant assay. It will be recognized that the selection of the parameters necessary for optimizing the performance of these methods are within the abilities of the ordinarily skilled artisan. Analysis may be performed using either direct immunochemistry (if the antibodies have been labeled with a detectable moiety) or by indirect immunochemistry.

Other embodiments of the current invention provide for pharmaceutical compositions comprising MTV DNA, RNA and/or proteins described *supra*. These compositions may further

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comprise a pharmaceutically acceptable excipient, carrier, or diluent and do not contain any biologically harmful substances. The pharmaceutical compositions of the present invention may be formulated by one having ordinary skill in the art. Suitable pharmaceutical formulations are described in *Remington's Pharmaceutical Sciences* which is a standard reference text in the field  
5 which is here incorporated by reference.

The pharmaceutical compositions may further comprise coloring or stabilizing agents, osmotic agents, antibacterial agents, or any other substances as long as such substances do not interfere with the function of the composition. The pharmaceutical compositions of the invention, can, for example, be formulated as a solution, suspension, or emulsion in association  
10 with a pharmaceutically acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human albumen. Liposomes may also be used. The vehicle may contain additives that maintain isotonicity (e.g., sodium chloride or mannitol) and chemical stability (e.g., buffers and preservatives). It should be appreciated that endotoxin contamination should be kept at a safe level, for example, less than 0.5 ng/mg protein.  
15 Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by the United States Food and Drug Administration Office of Biological Standards. The formulations may be sterilized by commonly used techniques such as filtration.

The phrase "pharmaceutically acceptable" refers to substances and compositions which  
20 do not produce an adverse, allergic, or otherwise untoward reaction when administered to an animal, or a human, as appropriate. A substance which caused produced any of these adverse effects would be classified as "biologically harmful" within the scope of the present invention. Pharmaceutically acceptable substances and compositions include, but are not limited to solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption  
25 delaying agents. Except where incompatible with the invention the use of any conventional ingredient is contemplated. Furthermore, supplementary active ingredients which serve some other pharmacologically expedient purpose can also be incorporated into the instant compositions.

The methods and compositions described above are contemplated to be of great benefit in  
30 helping to determine whether or not MTV or MTV viral components are present in a person's tissues. This knowledge is would be of aid in predicting a patient's susceptibility to cancer

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etiologically derived from MTV. The methods provide a means for determining a patient's viral load (the amount of virus present in the person's tissues). In this respect it is envisioned that tremendous benefit would be achieved by adapting the methods herein described to the widespread screening of the general population, and in particular to all mature human females.

5 Other compositions contemplated as part of various embodiments of the instant invention provide a means for stabilizing or reducing the amount of virus present in a human or other animal.

The various methods described above are readily adaptable to preparing diagnostic kits for detecting the presence of MTV DNA, RNA, and/or polypeptides. Consequently, for the clinical practice of the invention, yet other embodiments of the instant invention, which provide for diagnostic kits, are contemplated. Such kits are useful for the qualitative and quantitative analysis of a sample in order to detect, and perhaps determine the quantity of, MTV DNA, RNA, and/or polypeptide present therein. In one aspect of this embodiment of the invention the kit includes, as part of its components, one or more recombinant DNA molecules comprising one or more of the MTV DNA sequences described above. Also according to this aspect of the invention the kit may comprise, in addition to (or in alternative to) the recombinant MTV DNA, one or more synthetic oligonucleotide primer pairs useful for the PCR amplification of these MTV DNA sequences.

Another aspect of this embodiment of the invention provides for a kit for detecting antibodies which specifically recognize mammary tumor virus proteins. As part of its components this kit includes a reagent comprising one or more polypeptides encoded by the MTV DNA sequences described above.

Also contemplated are kits useful for detecting the presence of MTV proteins by immunocytochemistry which comprise one or more antibodies (either monoclonal or polyclonal) which are specific for at least one MTV polypeptide. Optionally, these antibodies may be modified so as to comprise a detection moiety (e.g., a fluorescent dye or a radioactive isotope, such as <sup>35</sup>S).

Kits according to these embodiments of the invention may comprise packages, each containing one or more of the various reagents (typically in concentrated form) which are required to perform the respective diagnostic tests. The kits according to these embodiments of the invention are contemplated to be useful for detecting and/or quantifying MTV DNA, RNA,

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and/or protein in biological (or other types of) samples. Such samples may be selected from, but are not limited to the following: blood plasma or serum, whole blood, urine, sputum, colonic effluent, cerebrospinal fluid, lymphatic fluid, bone marrow, tissue samples (such as from a surgical biopsy), or any other sample suspected of containing these biological molecules.

5       The kits also may further include one or more fiduciary results. As used herein a "fiduciary result" refers to a reference standard against which a test outcome is compared to gauge the results in terms of quality and/or quantity. A "fiduciary series" is a plurality of such references that represent points along a qualitative or a quantitative scale. Preferable in this regard, are kits that include a fiduciary series for interpreting results. The fiduciary may be in  
10 the form of one or more photographs or may be depicted in other ways, including written descriptions.

Thus fiduciaries may be developed as part of the kits, in accordance with this aspect of the invention, to guide interpretation of results. In this regard, a RNA or polypeptide concentration from a biological sample may be characterized in accordance with the foregoing.  
15 Sample may be taken from representative cross-section of patients at various stages of disease (including asymptomatic or essentially disease free) to prepare a fiduciary series. Characterization in this regard may benefit from hindsight, by following the actual course of neoplastic progression in patients as they undergo diagnosis, treatment, and follow up thereafter. The determination of viral load (as determined by the abundance of MTV DNA, RNA, or  
20 polypeptide), as set out above, for a variety of patients of known breast cancer status and eventual outcome, and the subsequent correlation of these values is of incalculable prognostic value. This will help provide the patient with a more accurate prognosis, and it will also aid the patient and oncologist in determining the best course of therapeutic treatment, when such treatment is necessary.

25       Another embodiment of the instant invention provides for a composition which induces an immunological response against MTV in an animal. A specifically contemplated aspect of this invention is a composition which induces an immunological response to human mammary tumor viral protein in humans (see Example 7). It is expected that such induced immunity may prevent breast cancer in individuals that carry endogenous MTV, by blocking the spread of the  
30 virus to hormonally sensitive tissues.

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Methods for producing an immune response to viral DNA and/or RNA in animals are also known, see for example U.S. patent numbers 5,990,091 and 6,004,799 which are herein incorporated by reference. Thus compositions which elicit an immune response to MTV DNA and/or RNA are also contemplated as aspect of this embodiment of the invention.

5 Other embodiments provide for therapeutic compositions which decrease the activity and presence of human mammary tumor virus. A common feature of all retroviruses is the presence of three enzymes involved in various stages of the viral replication cycle, the reverse transcriptase (RT), protease (PR), and integrase (IN). Various pharmaceuticals have been developed which target the RT and PR of human immunodeficiency virus (HIV), a retrovirus  
10 distantly related to MTV. Furthermore, chemicals which selectively inhibit HIV IN have been identified and prototype drugs targeting this enzyme are under development (Hong *et al.*, 1998; Mathe, 1999; Robinson, 1998; Singh *et al.*, 2000). According to one aspect of the instant invention a pharmaceutical composition comprising one or a mixture of two or more retroviral inhibitors in an amount effective to inhibit the activity or the spread of MTV's of the instant  
15 invention is provided.

Identification of the drug or drugs for use in the pharmaceutical compositions of this embodiment can be made using techniques known to those skilled in the art. For examples of protease and reverse transcriptase inhibitors and methods of determining the efficacy of such drugs as retroviral inhibitors see U.S. Patent Nos. 5,858,738, 6,017,928, and 6,046,228, which  
20 are herein incorporated by reference. The various compositions of this embodiment of the invention may comprise, for example, currently known HIV integrase, protease, or reverse transcriptase inhibitors; alternatively, such inhibitors can be modified so as to increase their specificity for MTV's. Likewise, other classes of HIV inhibitors, such as peptides which are analogs of the transmembrane glycoprotein, can serve as pro-drugs to develop specific drugs  
25 which decrease activity and presence of MTV in humans and other species. These compositions are contemplated as being useful to inhibit the activity and spread of MTV in humans and other species which carry these viruses as endogenous genetic elements. Such drugs could be used to treat breast cancer patients afflicted with MTV and would be expected to ameliorate the severity and to reduce the recurrence of disease. In addition, such drugs could be used prophylactically to  
30 prevent the occurrence of individuals at high risk for developing the disease.

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## EXAMPLES

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

### EXAMPLE 1: MMTV-related sequences in humans

Sequences which are highly similar (>95%) to the MMTV *env* gene were amplified by PCR from human DNA samples, including subsets of both BC (breast cancer) tissue and non-BC tissues. The MMTV-related sequences were found by PCR and a sensitive blotting technique not only in breast tumors (see Figure 1), but also in the blood of a subset of healthy controls (see Figure 2), and systemic lupus erythematosus (SLE) patients without breast cancer. Our results differ from those of Wang and coworkers (1995) who, with few exceptions, were able to detect MMTV-like sequences only in breast tumors. The sequences from human DNA were distinct from the MMTV sequences used as controls in these PCR reactions indicating that our results are not simply due to contamination. A ribonuclease protection assay was used to confirm these results using a non-PCR based technique to determine that the majority of the PCR positive BC tissues, but none of the PCR negative tissues, expressed this sequence at the mRNA level. Many of the products from these PCR reactions have been sequenced. Analysis of these sequences provides further strong evidence that PCR contamination is an unlikely explanation for the observed results. MMTV *env*-like sequences from different individuals derived in the same PCR run were distinct from each other. This result indicates the lack of an ubiquitous PCR contaminant that would have produced a more consistent sequence that should have been identical (or nearly so) in the various reaction tubes. Furthermore DNA of individual subjects produced internally consistent MMTV *env*-like sequences from PCR run to PCR run. The variations within the MMTV-related sequences from a given patient may represent a low number of Taq errors, but are also suggestive of variations expected of a replicating retrovirus (reverse transcriptase errors).

**Example 2: RNase Protection Assay determination of MTV RNA levels**

Ribonuclease protection assay (RPA) is a quantitative assay which is frequently used by those of ordinary skill in the art to determine the levels of specific RNA species without PCR amplification. Briefly, it is performed as follows for the transcripts of the present invention: a probe of uniform length is synthesized by *in vitro* transcription of a cloned template and labeled to high specific activity. <sup>[35]</sup>S-labeled riboprobes were prepared from plasmids containing the cloned MTV fragments in the 150 to 400 bp size range. The probe was then hybridized to test RNA, and RNA fragments that remain unhybridized are not protected from digestion by RNase A/T1. The fragments of the labeled riboprobe protected by hybridized RNA were visualized and analyzed following separation on a denaturing polyacrylamide gel. Levels of MTV-related mRNA are compared in each sample to levels of mRNA produced by the  $\beta$ -actin gene, a "housekeeping" gene to ensure integrity of the RNA sample (*i.e.*, that the RNA has not been degraded). RPA detected RNA in 2 out of 3 PCR positive breast tumors (see Figure 3). RPA is 10-15 times more sensitive than "northern" analysis for detection of rare messenger RNA. RPA analysis is performed directly on total RNA, without any prior manipulation, which can introduce errors in the quantitative analysis.

**Example 3: RT-PCR determination of MTV RNA levels**

MTV mRNAs may also be detected using real time RT-PCR (reverse transcriptase linked polymerase chain reaction). For example the I-Cycler (Biorad), with a fluoroscopic detection facility is capable of determining the real time kinetics of the PCR amplification product by quantifying the PCR product in the log-linear phase of the PCR reaction. Using this method, a real-time thermocycler can reliably compare miniscule amounts of known nucleotide template in a reproducible fashion.

**Example 4: Detection of anti-MTV antibodies in blood by Western blot analysis**

The InsectSelect<sup>TM</sup> System (Invitrogen) which allows stable production of recombinant proteins in insect cells in a manner similar to well-characterized baculovirus expression systems may be used to generate recombinant MTV proteins. This is a virus-free system which allows creation of stable cell lines that continuously produce high-quality protein. Stable cell lines generated in about 9 days may be used for continuous, long-term production of recombinant proteins. The InsectSelect<sup>TM</sup> expression system is based on a plasmid vector which carries an



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antibiotic resistance gene for selecting stably expressing insect cell lines. This expression vector uses the immediate early promoter, OpIE2, from the Douglas Fir Tussock moth OpMNPV baculovirus for gene expression. OpIE2 is a strong transcriptional promoter in lepidopteran (Sf9, 5121, High Five<sup>TM</sup> (Invitrogen)) as well as mosquito and dipteran cell lines. The expression vector pIZ/V5-His (Invitrogen) has a multiple cloning site and several features that simplify production and analysis of recombinant proteins in insect cells. It includes a Zeocin-resistance gene that allows for rapid selection of stably transfected cells, a C-terminal tag encoding the V5 epitope, and a C-terminal polyhistidine (6xHis) sequence. These latter features facilitate rapid protein detection with anti-V5 antibodies (Invitrogen) and protein purification with resins that bind the polyhistidine. Alternative eukaryotic expression systems for MMTV related proteins are an *in vitro* rabbit reticulocyte lysate system (Promega) with *in vitro* transcribed and capped mRNA or the Sindbis virus Expression system (Invitrogen).

MMTV-related genes may also be cloned into a pGEX vector (Pharmacia) and expressed in bacteria to make recombinant fusion protein with glutathione S-transferase (GST) protein for immunoblot studies. This system has several advantages compared to other methodologies of protein expression, not the least of which is the ease of production, isolation, and purification of the recombinant protein. Fusion proteins generated with the GST protein typically remain soluble permitting the recovery from cell lysates. Denaturing conditions are not required during purification and therefore the antigenic and enzyme properties of the protein are often maintained. Moreover, the GST fusion protein may be efficiently and rapidly purified by immobilizing the GST on glutathione coated beads or columns and eluted with reduced glutathione.

To perform a western immunoblot, MTV proteins are dissolved in lysis buffer (0.25 M Tris Base, pH 6.8, containing 4% sodium dodecyl sulfate, 10% dithiothreitol, 20% glycerol, and 0.01% w/v bromophenol blue), heated to 100°C for 3 min and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% polyacrylamide gel. These proteins may then be transferred electrophoretically to a nitrocellulose membrane (Protran<sup>TM</sup>; Schleicher & Schuell) in Tris-glycine (pH 8.3) buffer with 20% methanol. The blots may be incubated overnight in blocking buffer (0.02 M Tris, 0.1 M NaCl, heat inactivated goat serum, 0.01% thimerosal, and 5% nonfat dry milk) with serum or plasma from the human or animal being tested (1:100 dilution or optimal dilution). Incubation with secondary antibodies,

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biotinylated anti-human (or other species) IgG goat antibodies diluted 1:500 or 1:1,000 in blocking buffer, and with avidin-horseradish peroxidase may be performed at room temperature for 2 hours. The immunoblots may be developed with 7.8 mM 4-chloro-1-naphthol and 0.03% hydrogen peroxide. Bands corresponding to MTV proteins are quantitated by scanning and  
5 processed with image analysis software (NIH Image).

**Example 5: Detection of anti-MTV antibodies by enzyme linked immunoassay**

Antibodies which specifically bind to MTV protein(s) may be detected using an enzyme-linked immunoassay with MTV protein or proteins as the target for the antibodies. In this technique MTV proteins produced in an expression system, such as the baculovirus/insect cell  
10 clutter system or purified from virus preparations may be bound to the bottom of wells in a multiwell plastic microtiter plate. Serum or plasma from humans or other species is diluted to an empirically determined optimum dilution and incubated from 1 hour to overnight at about 25 °C (room temperature). The wells are then washed three times in a saline solution containing Tween® 20 (Aldrich) or NP-40 (currently available as Igepal™ CA-630, Sigma) detergents using  
15 an automated plate washer. Antibodies bound to the MTV proteins may then be detected by reacting the wells sequentially with buffers containing biotinylated goat anti-human immunoglobulin, followed by avidin coupled to horseradish peroxidase, and finally 3,3',5,5'-tetramethylbenzidine a substrate for horseradish peroxidase which produces a colored reaction product. Between each step the well is typically washed three times in a saline solution  
20 containing Tween® 20 or NP-40 detergents using an automated plate washer. The colored reaction produced in each well is quantitated using a spectrophotometer plate reader. The amount of colored reaction product is proportional to the amount of antibodies to MTV present in the original sample.

**Example 6: Detection of MTV retroviral proteins in situ using immunohistochemistry**

25 MTV retroviral proteins may be detected in tissue samples by immunohistochemical assays. In this technique MTV proteins produced in an expression system, such as the baculovirus/insect cell clutter system or purified from virus preparations may be used to generate monoclonal or polyclonal antibodies by methods well known to those skilled in the art. These antibodies may then be labeled with a detection moiety such as biotin, fluorescein, horseradish  
30 peroxidase or other labels used for this purpose. The labeled antibody preparations are then

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diluted to an empirically determined optimum dilution and incubated with fixed or frozen thin sections or cell preparations from samples to be tested for the presence of MTV proteins. The samples are processed, as dictated by the type of label used, and the binding of the antibody to MTV retroviral proteins may be detected by microscopy, autoradiography, flow cytometry, etc.,  
5 again, as dictated by the identity of the labeling moiety.

**Example 7: Preparation of a pharmaceutical composition capable of eliciting an immunological response, against human mammary tumor virus, in humans.**

A pharmaceutical composition to be used to induce an immunological response in humans or animals to MTV protein may be developed as follows. MTV proteins produced in an  
10 expression system, such as the baculovirus/insect cell culture system described above or purified from virus preparations may be extensively purified using column chromatography and/or any other methodologies commonly employed by skilled artisans. The purified MTV proteins may then be mixed or emulsified with a suitable adjuvant preparation (currently alum is the only adjuvant approved for use in humans) to produce a vaccine. This immunogenic composition  
15 may then be injected subcutaneously or intradermally into the target animal.

All of the composition and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred  
20 embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are  
25 deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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## CLAIMS:

1. An isolated DNA molecule comprising a DNA sequence that has:
  - a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or
  - 5 b) at least 99% identity with one or more of the following: at least 340 contiguous bases of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360 of SEQ ID NO:5, at  
10 least 130 contiguous nucleotides of SEQ ID NO:6, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:7, at least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170 contiguous nucleotides of SEQ ID  
15 NO:23, at least 100 contiguous nucleotides of the sequence represented by nucleotides 337-462 of SEQ ID NO:25, at least 300 contiguous nucleotides of the sequence represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or
  - c) at least 92% identity with SEQ ID NO:10.
- 20 2. The isolated DNA molecule of claim 1 which comprises SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 10, 21, 23, 25, 27, or 29.
3. The isolated DNA molecule of claim 1 wherein the DNA sequence is derived from human, rhesus macaque, or cat.
4. The isolated DNA molecule of claim 1, further comprising a detection moiety.
- 25 5. The isolated DNA molecule of claim 1, which is suspended or dissolved in a diluent.
6. The isolated DNA molecule of claim 5 wherein the diluent is a buffered aqueous solution.



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aic017. The isolated DNA molecule of claim 5 wherein the DNA is present at a concentration of from about 0.1 ng/ $\mu$ l to 100  $\mu$ g/ $\mu$ l.

8. A purified polypeptide encoded by a DNA molecule comprising a DNA sequence that has:

- 5 a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or
- b) at least 99% identity with one or more of the following: at least 340 contiguous bases of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360 of SEQ ID NO:5, at  
10 at least 130 contiguous nucleotides of SEQ ID NO:6, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:7, at least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170 contiguous nucleotides of SEQ ID  
15 NO:23, at least 100 contiguous nucleotides of the sequence represented by nucleotides 337-462 of SEQ ID NO:25, at least 300 contiguous nucleotides of the sequence represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or

20 c) at least 92% identity with SEQ ID NO:10.

9. The purified polypeptide of claim 8 which is SEQ ID NO: 12, 13, 14, 15, 16, 17, 18, 20, 22, 24, 26, 28, or 30.

ac0310. The purified polypeptide of claim 8 which is present in a pharmaceutical composition.

25 11. An antibody which recognizes a polypeptide encoded by a DNA molecule comprising a DNA sequence that has:

- a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or
- b) at least 99% identity with one or more of the following: at least 340 contiguous bases of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence represented by

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nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360 of SEQ ID NO:5, at least 130 contiguous nucleotides of SEQ ID NO:6, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:7, at least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170 contiguous nucleotides of SEQ ID NO:23, at least 100 contiguous nucleotides of the sequence represented by nucleotides 337-462 of SEQ ID NO:25, at least 300 contiguous nucleotides of the sequence represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or

c) at least 92% identity with SEQ ID NO:10;  
wherein said antibody was generated using said polypeptide.

12. The antibody of claim 11 which is a monoclonal antibody.

13. An RNA corresponding to a DNA molecule a DNA molecule comprising a DNA sequence that has:

a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or

b) at least 99% identity with one or more of the following: at least 340 contiguous bases of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360 of SEQ ID NO:5, at least 130 contiguous nucleotides of SEQ ID NO:6, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:7, at least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170 contiguous nucleotides of SEQ ID NO:23, at least 100 contiguous nucleotides of the sequence represented by nucleotides 337-462 of SEQ ID NO:25, at least 300 contiguous nucleotides of the

- 32 -

sequence represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or

c) at least 92% identity with SEQ ID NO:10.

14. The isolated DNA molecule of claim 1 which is incorporated in a vector;  
5 wherein said DNA sequence is under the transcriptional control of a heterologous promoter.

15. The vector of claim 14 which is capable of expressing the DNA sequence in at least one of the following cell types: insect cells, bacterial cells, avian cells, yeast cells, or mammalian cells.

10 16. The vector of claim 14 wherein said vector is capable of episomal replication or chromosomal integration in at least one of the following cell types: insect cells, bacterial cells, avian cells, yeast cells, or mammalian cells.

17. A method for the detecting the presence of a mammary tumor viral DNA comprising the steps of:

15 i) obtaining a biological sample suspected of containing DNA which comprises a sequence molecule a DNA molecule comprising a DNA sequence that has:  
a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or  
b) at least 99% identity with one or more of the following: at least 340 contiguous  
20 bases of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360 of SEQ ID NO:5, at least 130 contiguous nucleotides of SEQ ID NO:6, at least  
25 140 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:7, at least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170 contiguous nucleotides of SEQ ID NO:23, at least 100 contiguous nucleotides of the sequence represented by nucleotides 337-462 of SEQ ID NO:25, at least 300

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contiguous nucleotides of the sequence represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or

- c) at least 92% identity with SEQ ID NO:10;
- ii) carrying out a polymerase chain reaction to amplify the suspected DNA sequence as defined in step i); and,
- iii) determining the sequence of, or otherwise characterizing, the amplicons produced in step ii) to determine whether or not the DNA sequence as defined in step i) is present in the sample.

18. The method of claim 17 wherein the biological sample is obtained from human, rhesus

macaque, or cat. 19. A method of detecting the presence of antibodies which recognize one or more mammary tumor virus polypeptides, the method comprising the steps of:

- i) obtaining a sample suspected of containing antibodies specific for mammary tumor viral antibodies;
- ii) obtaining at least one purified polypeptide encoded by a DNA molecule comprising a DNA sequence that has:
  - a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or
  - b) at least 99% identity with one or more of the following: at least 340 contiguous bases of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360 of SEQ ID NO:5, at least 130 contiguous nucleotides of SEQ ID NO:6, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:7, at least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170 contiguous nucleotides of SEQ ID NO:23, at least 100 contiguous nucleotides of the sequence represented by nucleotides 337-462 of SEQ ID NO:25, at least 300 contiguous nucleotides of the sequence

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represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or

c) at least 92% identity with SEQ ID NO:10;

iii) performing an immunochemical analysis using the sample of step i) and the polypeptide of step ii); and,

iv) analyzing the results of step iii) to determine whether or not antibodies which specifically interact with the peptide of step ii) are present in the sample.

20. The method of claim 19 wherein the immunochemical analysis of step iii) is a Western blot analysis.

21. The method of claim 19 wherein the immunochemical analysis of step iii) is enzyme-linked immunosorbant assay (ELISA) analysis.

22. The method of claim 19 wherein one or more of the polypeptides are derived from the *env*, or *gag*, gene and the antibodies are specific for one or more of the following polypeptides SEQ ID NO: 12, 13, 14, 15, 16, 17, 18, 20, 22, 24, 26, 28, or 30.

23. The method of claim 19 wherein the sample of step ii) comprises antibodies derived from a human, rhesus macaque, or cat.

24. A diagnostic kit for detecting DNA or RNA from a mammary tumor virus, said kit comprising a reagent of a DNA molecule that comprising a DNA sequence that has:

a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or

b) at least 99% identity with one or more of the following: at least 340 contiguous bases of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360 of SEQ ID NO:5, at least 130 contiguous nucleotides of SEQ ID NO:6, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:7, at least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170 contiguous nucleotides of SEQ ID NO:23, at least 100 contiguous nucleotides of the sequence represented by

- 35 -

nucleotides 337-462 of SEQ ID NO:25, at least 300 contiguous nucleotides of the sequence represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or

c) at least 92% identity with SEQ ID NO:10.

- 5 25. A diagnostic kit for detecting antibodies to mammary tumor virus, where said kit comprises a reagent comprises one or both of the following:
- i) one or more polypeptides encoded by a DNA molecule comprising a DNA sequence that has:
- a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or
- 10 b) at least 99% identity with one or more of the following: at least 340 contiguous bases of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360
- 15 of SEQ ID NO:5, at least 130 contiguous nucleotides of SEQ ID NO:6, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:7, at least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170
- 20 contiguous nucleotides of SEQ ID NO:23, at least 100 contiguous nucleotides of the sequence represented by nucleotides 337-462 of SEQ ID NO:25, at least 300 contiguous nucleotides of the sequence represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or
- c) at least 92% identity with SEQ ID NO:10; or
- 25 ii) an antibody specific for a polypeptide according to step i).
26. A method for the detection of MTV RNA in a sample which comprises the following steps:
- i) obtaining an sample suspected of containing RNA which encoded by one or more DNA sequences that have:
- 30 a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or

- 36 -

- 5 b) at least 99% identity with one or more of the following: at least 340 contiguous bases of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360 of SEQ ID NO:5, at least 130 contiguous nucleotides of SEQ ID NO:6, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:7, at least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170 contiguous nucleotides of SEQ ID NO:23, at least 100 contiguous nucleotides of the sequence represented by nucleotides 337-462 of SEQ ID NO:25, at least 300 contiguous nucleotides of the sequence represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or
- 10 c) at least 92% identity with SEQ ID NO:10;;
- 15 iv) carrying out an RNase protection assay (RPA); and,
- iii) analyzing the RPA results to determine whether RNA as defined in step i) is present in the sample and optionally quantitating said RNA.
- 20

1/3

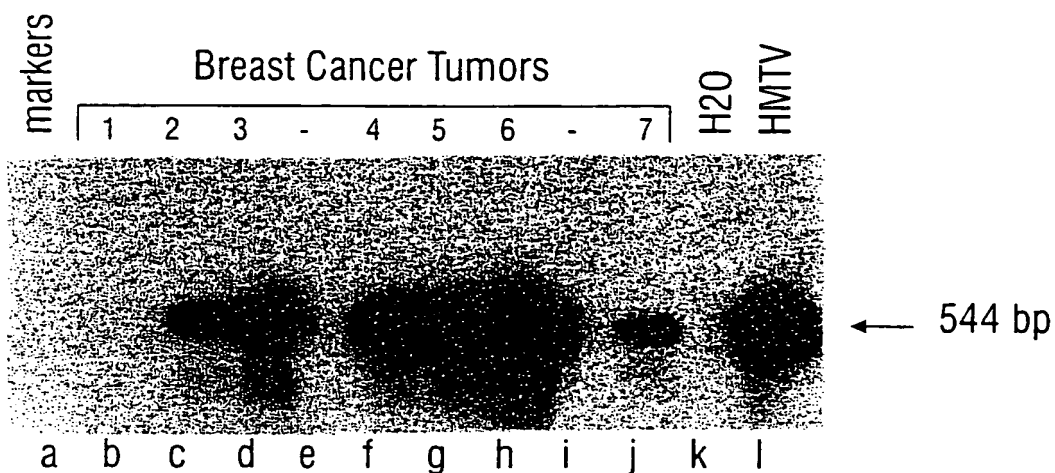


FIG. 1A

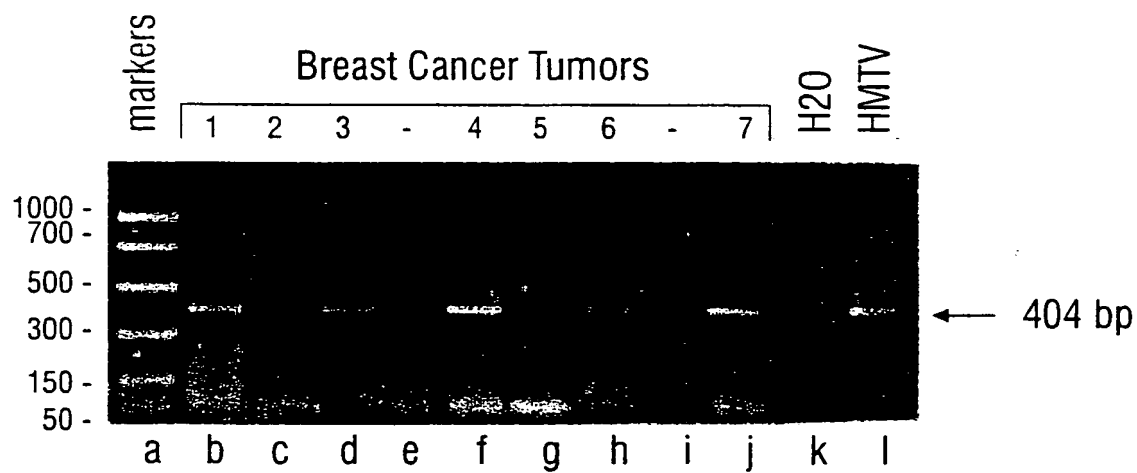


FIG. 1B



2/3

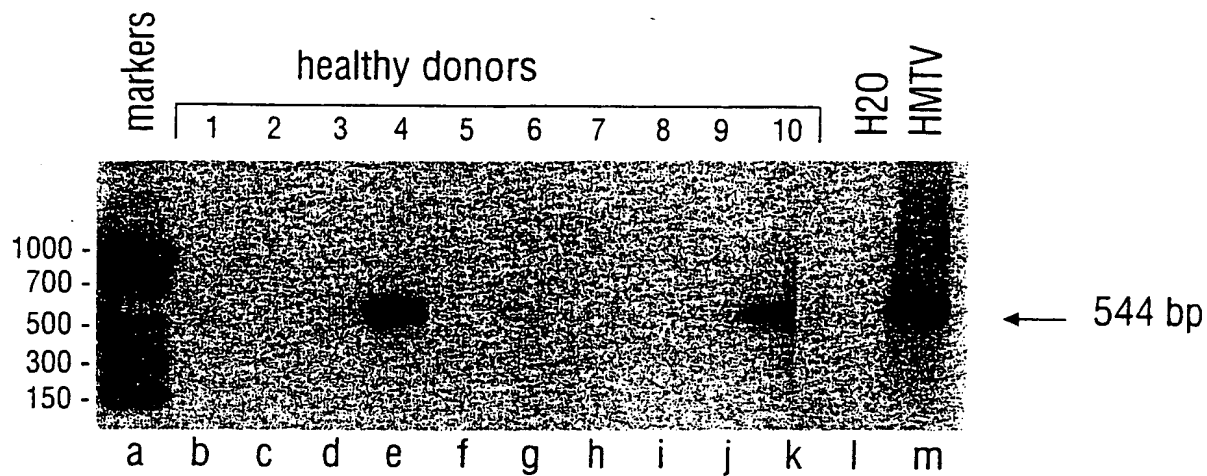


FIG. 2A

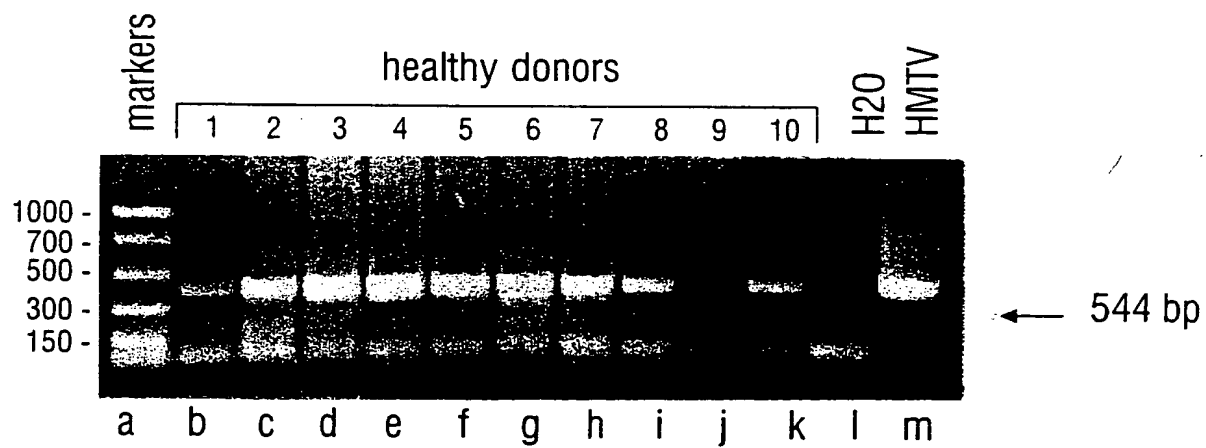


FIG. 2B

3/3

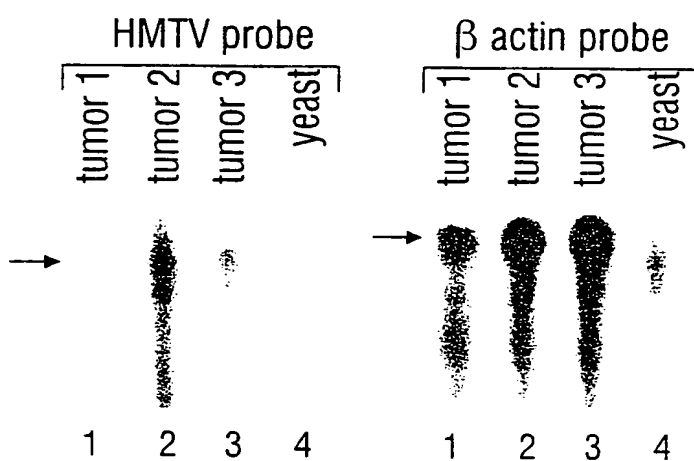


FIG. 3

- 1 -

## SEQUENCE LISTING

&lt;110&gt; Garry, Robert F.

5 &lt;120&gt; Human Endogenous Retrovirus in Breast Cancer

&lt;130&gt; TUMC:012

&lt;140&gt;

10 &lt;141&gt;

&lt;160&gt; 30

&lt;170&gt; PatentIn Ver. 2.1

15

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&lt;212&gt; DNA

&lt;213&gt; Mouse mammary tumor virus

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30

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&lt;211&gt; 461

&lt;212&gt; DNA

10 &lt;213&gt; Human Mammary Tumor Virus

&lt;400&gt; 4

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25 &lt;212&gt; DNA

&lt;213&gt; Rhesus Mammary Tumor Virus

&lt;400&gt; 5

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40 &lt;211&gt; 461

&lt;212&gt; DNA

&lt;213&gt; Rhesus Mammary Tumor Virus

&lt;400&gt; 6

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- 3 -

## &lt;213&gt; Cat Mammary Tumor Virus

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10 atttcctgtt cttctttag attgactaat tgtttagatt cttctgccta cgactatgca 420
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&lt;210&gt; 8

15 &lt;211&gt; 461

&lt;212&gt; DNA

&lt;213&gt; Cat Mammary Tumor Virus

&lt;400&gt; 8

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30 &lt;210&gt; 9

&lt;211&gt; 104

&lt;212&gt; DNA

&lt;213&gt; Mouse mammary tumor virus

35 &lt;400&gt; 9

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40 &lt;210&gt; 10

&lt;211&gt; 104

&lt;212&gt; DNA

&lt;213&gt; Cat Mammary Tumor Virus

45 &lt;400&gt; 10

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50 &lt;210&gt; 11

&lt;211&gt; 153

&lt;212&gt; PRT

&lt;213&gt; Mouse mammary tumor virus

55 &lt;400&gt; 11

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- 4 -

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15 Arg Pro Gly Phe Gln Glu His Asp Met Ile Pro Thr Ser Ala Cys Val  
                   85                  90                  95

Thr Tyr Pro Tyr Ala Ile Leu Leu Gly Leu Pro Gln Leu Ile Asp Ile  
                   100                  105                  110

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                   20                  25                  30

Arg Trp Tyr Glu Ala Gly Trp Val Glu Pro Thr Trp Phe Trp Glu Asn  
                   35                  40                  45

45 Ser Pro Lys Asp Pro Asn Asp Arg Asp Phe Thr Ala Leu Val Pro His  
           50                  55                  60

Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg Tyr Leu Ile Leu Lys  
       65                  70                  75                  80

50 Arg Pro Gly Phe Gln Glu His Asp Met Ile Pro Thr Ser Ala Cys Val  
                   85                  90                  95

55 Thr Tyr Pro Tyr Ala Ile Leu Leu Gly Leu Pro Gln Leu Ile Asp Ile  
                   100                  105                  110

Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Arg Leu

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 Thr Asn Cys Leu Asp Ser Ser Ala Tyr Asp Tyr Ala Ala Ile Ile Val  
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                                     20                                      25                                      30  
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                                     35                                      40                                      45  
 25 Ser Pro Lys Asp Pro Asn Asp Arg Asp Phe Thr Ala Leu Val Pro His  
         50                                      55                                      60  
     Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg His Leu Ile Leu Lys  
         65                                      70                                      75                                      80  
 30 Lys Pro Gly Phe Gln Glu His Glu Met Ile Pro Thr Ser Ala Cys Val  
                                     85                                      90                                      95  
     Thr Tyr Pro Tyr Ala Ile Leu Leu Gly Leu Pro Gln Leu Ile Asp Ile  
         100                                      105                                      110  
 35 Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Leu Thr  
         115                                      120                                      125  
 40 Asn Cys Leu Asp Ser Ser Ala Tyr Asp Tyr Ala Ala Ile Ile Val Lys  
         130                                      135                                      140  
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         145                                      150  
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     <400> 14  
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                                     20                                      25                                      30

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 85 90 95  
 Thr Tyr Pro Tyr Ala Ile Leu Leu Gly Leu Pro Gln Leu Ile Asp Ile  
 15 100 105 110  
 Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Arg Leu  
 115 120 125  
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 Lys Arg Pro Pro Tyr Val Leu Leu Pro  
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 45 Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg His Leu Ile Leu Lys  
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 Lys Pro Gly Phe Gln Glu His Glu Met Ile Pro Thr Ser Ala Cys Val  
 85 90 95  
 50 Thr Tyr Pro Tyr Ala Ile Leu Leu Gly Leu Pro Gln Leu Ile Asp Ile  
 100 105 110  
 Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Arg Leu  
 55 115 120 125  
 Thr Asn Cys Leu Asp Ser Ser Ala Tyr Asp Tyr Ala Ala Ile Ile Val



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 Lys Arg Pro Pro Tyr Val Leu Leu Pro  
 145                                      150  
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                                     20                                      25                                      30  
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                                     35                                      40                                      45  
 Ser Pro Lys Asp Pro Asn Asp Arg Asp Phe Thr Ala Leu Val Pro His  
 50                                      55                                      60  
 25 Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg His Leu Ile Leu Lys  
 65                                      70                                      75                                      80  
 Lys Pro Gly Phe Gln Glu His Glu Met Ile Pro Thr Ser Ala Cys Val  
 85                                      90                                      95  
 30 Thr Tyr Pro Tyr Ala Ile Leu Leu Gly Leu Pro Gln Leu Ile Asp Ile  
 100                                      105                                      110  
 35 Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Arg Pro  
 115                                      120                                      125  
 Thr Asn Cys Leu Asp Ser Ser Ala Tyr Asp Tyr Ala Ala Ile Ile Val  
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 40 Lys Arg Pro Pro Tyr Val Leu Leu Pro  
 145                                      150  
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 35                                      40                                      45

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Ser Pro Lys Asp Pro Asn Asp Arg Asp Phe Thr Ala Leu Val Pro His  
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5 Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg His Leu Ile Leu Lys  
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Lys Pro Gly Phe Gln Glu His Lys Met Ile Pro Thr Ser Ala Cys Val  
 85 90 95

10 Thr Tyr Pro Tyr Ala Ile Leu Leu Arg Leu Pro Gln Leu Ile Asp Ile  
 100 105 110

15 Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Arg Leu  
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Thr Asn Cys Leu Asp Ser Ser Ala Tyr Asp Tyr Ala Ala Ile Ile Val  
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<400> 18

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 35 40 45

40 Ser Pro Lys Asp Pro Asn Asp Arg Asp Phe Thr Ala Leu Val Pro His  
 50 55 60

Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg His Leu Ile Leu Lys  
 65 70 75 80

45 Lys Pro Gly Phe Gln Glu His Lys Met Ile Pro Thr Ser Ala Cys Val  
 85 90 95

Thr Tyr Pro Tyr Ala Ile Leu Leu Arg Leu Pro Gln Leu Ile Asp Ile  
 100 105 110

50 Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Arg Leu  
 115 120 125

Thr Asn Cys Leu Asp Ser Ser Ala Tyr Asp Tyr Ala Ala Ile Ile Val  
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55 Lys Arg Pro Pro Tyr Val Leu Leu Pro

- 9 -

145

150

&lt;210&gt; 19

5 &lt;211&gt; 35

&lt;212&gt; PRT

&lt;213&gt; Mouse mammary tumor virus

&lt;400&gt; 19

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Trp Glu Lys Cys Lys Phe Ile Val Ser Gly Ser His Pro Pro Asn Thr  
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Lys Asn Arg  
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&lt;211&gt; 35

&lt;212&gt; PRT

&lt;213&gt; Cat Mammary Tumor Virus

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Lys Asn Arg  
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35

&lt;210&gt; 21

&lt;211&gt; 460

&lt;212&gt; DNA

&lt;213&gt; Human Mammary Tumor Virus

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&lt;400&gt; 21

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 cgatcatagt caagaaggcg ccatatgtgc tgctacctgt 460

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&lt;210&gt; 22

&lt;211&gt; 38

&lt;212&gt; PRT

55 &lt;213&gt; Human Mammary Tumor Virus

&lt;400&gt; 22

- 10 -

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 10  
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 gccatattat taggattacc tcagctaata gatatagaaa aaagaggatc tacttttcat 360  
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 45 Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg Tyr Leu Ile Leu Lys  
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 Arg Pro Gly Phe Gln Glu His Asp Met Ile Pro Thr Ser Ala Cys Val  
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 50 Thr Tyr Pro Tyr Ala Ile Leu Leu Gly Leu Pro Gln Leu Ile Asp Ile  
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 Thr Asn Cys Leu Asp Ser Ser Ala Tyr Asp Tyr Ala Ala Ile Ile Val

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35 40 45  
Ser Pro Lys Asp Pro Asn Asp Arg Asp Phe Thr Ala Leu Val Pro His  
50 55 60  
40 Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg Tyr Leu Ile Leu Lys  
65 70 75 80  
Arg Pro Gly Phe Gln Glu His Asp Met Ile Pro Thr Ser Ala Cys Val  
45 85 90 95  
Thr Tyr Pro Tyr Ala Ile Leu Leu Gly Leu Pro Gln Leu Ile Asp Ile  
100 105 110  
50 Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Arg Leu  
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145 150

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 aggccaggat ttcaagaaca tgacatgatt cctacatctg cctgtgttac ttacccttat 300  
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 20 <211> 152  
 <212> PRT  
 <213> Human Mammary Tumor Virus

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 30 Arg Trp Tyr Glu Ala Gly Trp Val Glu Pro Thr Trp Phe Trp Glu Asn  
 35 40 45  
 Ser Pro Lys Asp Pro Asn Asp Arg Asp Phe Thr Ala Leu Val Pro His  
 35 50 55 60  
 Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg Tyr Leu Ile Leu Lys  
 65 70 75 80  
 40 Arg Pro Gly Phe Gln Glu His Asp Met Ile Pro Thr Ser Ala Cys Val  
 85 90 95  
 Thr Tyr Pro Tyr Ala Ile Leu Leu Gly Leu Pro Gln Leu Ile Asp Ile  
 100 105 110  
 45 Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Arg Leu  
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55 <210> 29  
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&lt;212&gt; DNA

&lt;213&gt; Human Mammary Tumor Virus

&lt;400&gt; 29

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15 &lt;210&gt; 30

&lt;211&gt; 43

&lt;212&gt; PRT

&lt;213&gt; Human Mammary Tumor Virus

20 &lt;400&gt; 30

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Ser Leu Pro Ser Pro Ser Val Asp Gln Ser Asp Gln Ile Lys Asn Lys
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Lys Asp Leu Phe Gly Asn Tyr Thr Pro Pro Val Asn Lys Glu Val His
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Arg Trp Tyr Glu Ala Gly Trp Val Glu Pro Thr
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30





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New Orleans, LA 70112 (US).

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LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,  
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,  
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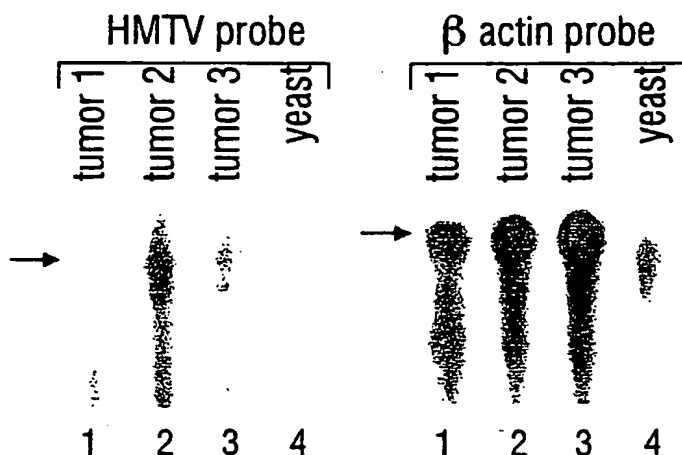
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For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: HUMAN ENDOGENOUS RETROVIRUS IN BREAST CANCER



(57) Abstract: The present invention is related to mammary tumor virus (MTV). MTV represents a group of retroviruses which possess very high homology to mouse mammary tumor virus (MMTV), a virus known to cause neoplastic mammary disease in mice. As described herein, MTV's have been identified in human, cat, and Rhesus macaque. The present invention specifically provides for recombinant nucleic acids and polypeptides derived from these MTV's as well as methods for using these biological molecules.



(19) World Intellectual Property Organization  
International Bureau



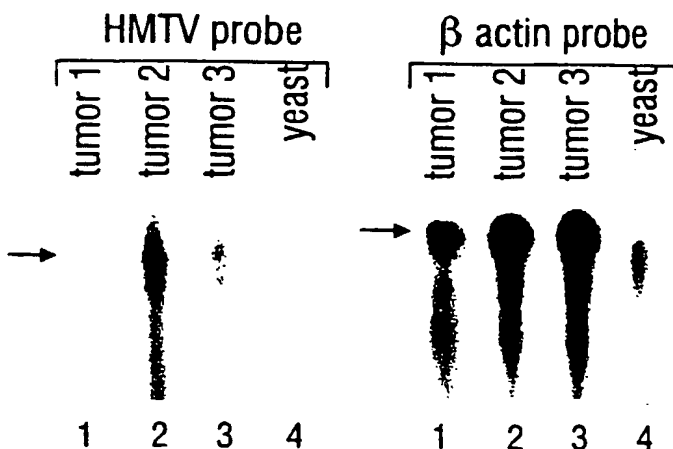
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(54) Title: HUMAN ENDOGENOUS RETROVIRUS IN BREAST CANCER



(57) Abstract: The present invention is related to mammary tumor virus (MTV). MTV represents a group of retroviruses which possess very high homology to mouse mammary tumor virus (MMTV), a virus known to cause neoplastic mammary disease in mice. As described herein, MTV's have been identified in human, cat, and Rhesus macaque. The present invention specifically provides for recombinant nucleic acids and polypeptides derived from these MTV's as well as methods for using these biological molecules.

# INTERNATIONAL SEARCH REPORT

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## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C07K14/47 C07K16/18 C12Q1/68 G01N33/566  
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## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, EMBL, BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 17470 A (HOLLAND JAMES F) 15 May 1997 (1997-05-15) the whole document	1-26
X	WANG Y. ET AL.: "Detection of mammary tumor virus env gene-like sequences in human breast cancer." CANCER RES., vol. 55, 15 November 1995 (1995-11-15), pages 5173-5179, XP002150741 cited in the application the whole document	1-26

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
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- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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12 January 2001

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# INTERNATIONAL SEARCH REPORT

In tional Application No

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WANG Y. ET AL.: "Expression of mouse mammary tumor virus-like env gene sequences in human breast cancer." CLIN. CANCER RES., vol. 4, no. 10, October 1998 (1998-10), pages 2565-2568, XP000953033 the whole document</p> <p style="text-align: center;">---</p>	1-26
X	<p>REDMOND S.M.S. &amp; DICKSON C.: "Sequence and expression of the mouse mammary tumor virus env gene." EMBO J., vol. 2, 1983, pages 125-131, XP000971882 compare Fig 2 with seq. 5,6,7,8,10.</p> <p style="text-align: center;">---</p>	1-26
A	<p>FAFF O. ET AL.: "Retrovirus-like particles from the human T47D cell line are related to mouse mammary tumor virus and are of human endogenous origin." J. GEN. VIROL., vol. 73, 1992, pages 1087-1097, XP002157129 the whole document</p> <p style="text-align: center;">-----</p>	1-26

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 00/18279

## B x I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-26, partly

An isolated molecule comprising a DNA sequences that has at least 99% identity with seq. IDs 2,3,4,21,23,25,27 or 29 or fragments thereof.

Corresponding compositions, polypeptides, antibodies, RNAs, vectors, methods of detection, diagnostic kits.

2. Claims: 1-26, partly

Idem as subject-matter 1 but limited to sequence IDs 5,6.

3. Claims: 1-26, partly

Idem as subject-matter 1 but limited to sequence IDs 7,8,10.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

In International Application No

PCT/US 00/18279

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9717470 A	15-05-1997	US 5686247 A	11-11-1997
		AU 717291 B	23-03-2000
		AU 1271497 A	29-05-1997
		EP 0866878 A	30-09-1998
		JP 2000500650 T	25-01-2000
		US 6040146 A	21-03-2000

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Form PCT/ISA/210 (patent family annex) (July 1992)



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NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,  
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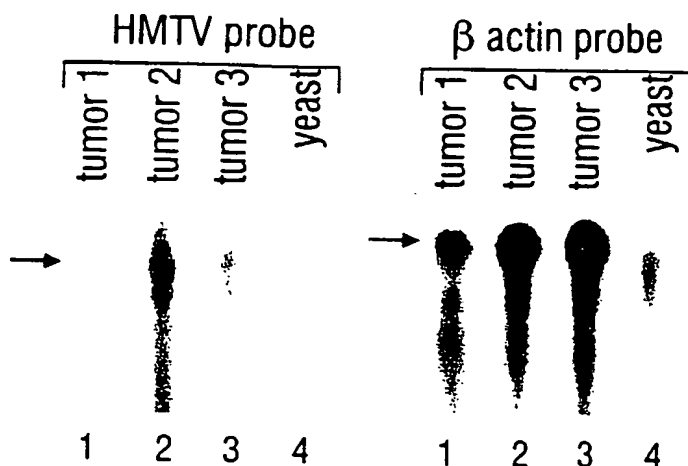
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- 1 -

## HUMAN ENDOGENOUS RETROVIRUS IN BREAST CANCER

### BACKGROUND OF THE INVENTION

The present application takes priority from Provisional U.S. patent application number 60/141,626 filed on June 30, 1999 the entire text of which is specifically incorporated herein by  
5 reference.

#### 1. FIELD OF THE INVENTION

The present invention relates to compositions and methods for the detection, prognostic evaluation, and treatment of oncogenic disorders, particularly breast cancer.

Specifically, the instant invention provides for compositions, useful for identifying and  
10 treating disorders related to newly identified endogenous retroviruses which are present in a subset of humans, cats, and nonhuman primates.

#### 2. TECHNICAL PROBLEM ADDRESSED BY THE INVENTION

##### **Mutations in known susceptibility genes do not account for all breast cancer**

Breast cancer (BC) is one of the leading causes of cancer death among women. The  
15 induction of BC is thought to involve the interplay of several factors, including the genetic, hormonal, immunological and physiological status of the host, as well as dietary habits and exposures to chemicals, radiation or infectious agents. It is now clear that variations of several genes, including BRCA-1 and BRCA-2, can result in greatly increased risks for development of BC. However, defects in known BC susceptibility genes account for only about 5% of BC, the  
20 so-called familial cases (Armstrong *et al.*, 2000; Gayther *et al.*, 1998).

As with other types of cancer, the possibility that a virus is etiologically involved in sporadically occurring BC has not been eliminated. Consequently, there has long been a need to determine which, if any viruses are causally linked to the development of BC. The identification of such a virus would likely provide invaluable aid in the following areas BC medicine:  
25 prevention, diagnosis, determination of prognosis; and treatment.

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### 3. DESCRIPTION OF RELATED ART

#### Retrovirus induction of breast cancer in mice

Mouse mammary tumor virus (MMTV), a B-type retrovirus, was discovered during studies of hereditary cancer in mice at the Jackson Laboratories in the 1930's (Bittner, 1936). As the prototype of slow-transforming retroviruses, MMTV has been definitively shown to cause BC in mice. Prior studies established that MMTV is transmitted both in the germline as endogenous proviruses and exogenously via milk. As endogenous elements MMTV proviruses follow patterns of Mendelian inheritance, as other sequences in the genome (Cohen *et al.*, Cell, 1979; Cohen and Varmus, 1979, 1980; Traina *et al.*, 1981; Traina-Dorge and Cohen, 1983; Traina-Dorge *et al.*, 1985; Varmus *et al.*, 1978). Horizontal transmission of MMTV typically occurs by infection of mouse pups by MMTV virions present in the milk of infected dams. Thus, it is possible to transmit MMTV to mice by foster feeding. 30 or more unique proviral integration sites for endogenous MMTV have been identified. However, some wild mice do not carry any endogenous MMTV proviruses (Cohen and Varmus, 1979; Cohen *et al.*, 1982). This result suggests that the many endogenous MMTV proviruses are relatively recent additions to the mouse genome. The most likely explanation is that MMTV entered the germline of certain mice (but not others) on multiple occasions after the evolutionary splits among the various species and subspecies of the genus *Mus*. Certain endogenous MMTV can be activated by hormones to form infectious virions capable of inducing mammary carcinomas after long latency periods. Most endogenous MMTV proviruses are defective and do not encode for infectious virions.

#### Roles of MMTV genes and cellular genes in oncogenesis

The MMTV Orf protein can function as a superantigen (SA). When expressed in the thymus during fetal/early development it can mediate complete or incomplete deletion of SA-reactive T-cells. SA expression is required to activate B-cells targets of MMTV in the gut-associated lymphoid tissue of nursing pups. Complete deletion of the SA responsive clones thus renders the mice resistant to MMTV infection in the gut and thereby leads to a low incidence of MMTV-induced tumors. On the other hand, in mice with only partially deleted responsive clones of lymphoid cells the SA activation stimulates expansion of the targets and spread of MMTV. As female infected animals reach puberty, estrogenic hormones drive expression of the MMTV long terminal repeat (LTR) through its hormone response element

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(HRE). This permits production and assembly of MMTV and spread of the virus to other hormonally-sensitive tissues, including the breast and ovaries. Integration of MMTV LTRs adjacent to certain cellular genes, such as the proto-oncogenes *Int*, *Wnt* and *Fgf*, can increase expression of these genes resulting in BC and other cancers.

5 The molecular genetic interactions between MMTV, the immune system of its murine host, and the breast and other hormonally-sensitive cells malignantly transformed by this retrovirus have been extensively studied. MMTV promotes mammary gland cancer in mice by insertional mutagenesis (Varmus *et al.*, 1978; Varmus, 1985). MMTV proviral LTR elements direct steroid hormone-dependent transactivation of various cellular oncogenes including *Wnt*,  
10 *Fgf* and *Int* thereby promoting clonal expansion of tumor cells (Shackleford and Varmus, 1987, Shackleford *et al.*, 1993; Jakobovits *et al.*, 1986; Nusse, 1991; Nusse *et al.*, 1985). For productive, persistent infection and completion of its replication cycle, MMTV must contain a superantigen and interact with a functional host immune system (Golovkina *et al.*, 1995; Luther and Acha-Orbea, 1996; Coffin, 1992).

#### 15 The search for a human breast cancer virus

The discovery of the oncogenic MMTV has prompted many investigators to explore a retroviral etiology for BC in humans (Sarkar, 1980). Data collected over the past five decades has suggested the existence of a human homologue of MMTV. In 1971, Moore and associates reported that 60% of human milk samples from BC patients contain B-type particles  
20 indistinguishable from MMTV by electron microscopy, compared to 5% of the general population (Moore *et al.*, 1971). These investigators also reported that 39% of Parsi women of India, an inbred population with a two-fold increased incidence of BC, had B-type particles in their milk (Das *et al.*, 1972; Moore, 1971). Several studies have demonstrated that BC cells, but not cells from normal tissues, also contain reverse transcriptase (RT), an enzyme associated with  
25 all retroviruses. Numerous investigators have examined serum and breast milk for the presence of antibodies reactive with MMTV. Most of these studies were performed in the pre-AIDS era, prior to the advent of highly sensitive and specific techniques for detecting anti-retroviral antibodies made necessary for detection of HIV antibodies in donated blood.

Despite the numerous electron microscopic, biochemical and immunological studies on  
30 human breast carcinoma tissue, milk, patients' sera, and breast carcinoma cell lines suggesting

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the existence of a human homologue of MMTV, proof that such an agent exists has remained elusive (Andersson *et al.*, 1996; Ziegler, 1997). Most authors have dismissed the importance of prior studies purporting to show evidence of a human homologue of MMTV because of the presence of numerous human endogenous retroviruses (HERVs) (Larsson *et al.*, 1994; Li *et al.*, 1996; Lower *et al.*, 1996; Meese *et al.*, 1996; Ono, 1986; Patience *et al.*, 1996; Faff *et al.*, 1992). There are about 50,000 HERVs or HERV-related sequences in the human genome, some of which have been shown to have up to 60% homology to MMTV. In this regard, it is important to note that seroreactivity to HERV-K10, to this point the HERV considered to be most closely related to MMTV, cannot account for MMTV-reactive antibodies present in the sera of breast cancer patients and the smaller number of healthy individuals (Vogetseder *et al.*, 1995). Furthermore, we believe that the presence of these MMTV-related sequences is precisely the reason that human homologues of MMTV have not previously been demonstrated conclusively by molecular techniques. The presence of these related, but distinct, sequences could have obscured the detection of more closely related sequences by prior investigators who used less sensitive techniques, such as Southern blotting.

Only recently have sequences with relatively high homology (>90%) to those of MMTV been isolated from human BC tissue (Wang *et al.*, 1995, 1998; ). Sequences 95 - 99% similar to MMTV *env* were amplified by PCR in 121 (38.5%) of 314 unselected breast cancer tumor samples. It is pertinent to note that the MMTV-like sequences were detected in only 2 (1.8%) of 107 breast specimens from reduction mammoplasties and in 0/80 samples from normal tissues or non-breast tumors. The MMTV-*env* like RNA was expressed (as determined by RT-PCR) in 66% of DNA PCR positive breast tumors (Wang *et al.*, 1998). A complete 9.9 kb provirus with 94% similarity to MMTV was detected in 2 breast tumors. FISH (fluorescence *in situ* hybridization) revealed integration at several sites in DNA derived from BC tumors, but not normal breast cells (Wang *et al.*, 1999 ACR mtg. abstracts #2933, 2944). Wang *et al.* suggested the existence of a human mammary tumor virus (HMTV) that is spread by the exogenous route of infection (horizontal transmission). Attempts by these and other investigators to amplify other regions of MMTV-related viruses, from the genomic DNA or cDNA of subjects who did not have BC, yielded HERV sequences (such as HERV-K10) with only about 60% homology to MMTV. Thus, BC tissues are the only tissues in which sequences that are highly similar to those

- 5 -

of MMTV have been heretofore found. Consequently normal breast tissue and other tissues appeared to be negative for the expression of viruses with high homology to MMTV.

#### **MMTV can be transmitted horizontally or vertically**

The retrovirus replication cycle is characterized by conversion of the single-stranded RNA viral genome into doubled-stranded proviral DNA by the multiple enzymatic activities of the virion-associated reverse transcriptase (RT). As is the case with other retroviruses, integration of MMTV proviral DNA into the genome of host cells is required for expression of viral proteins and production of infectious progeny. In both infected mouse mammary glands as well as heterologous cells MMTV proviral DNA is integrated into a large number of apparently random sites. Integration of MMTV proviruses containing transcriptionally active LTR near some cellular genes (proto-oncogenes), such as *Int*, *Wnt*, and *Fgf* can result in over-expression of these genes, cellular transformation and clonal expansion of the tumor cells (Varmus, 1985; Shackleford *et al.*, 1993; Jakobovits *et al.*, 1986; Cohen, 1980; Breznik and Cohen, 1982). The long latency of MMTV-induce carcinogenesis is explained in part by the necessity for proviruses to integrate into these particular sites.

Genetic differences among viral strains of MMTV can account in part for the varying incidence of BC in diverse strains of mice. Mice of the C3H strain have a greater than 90% incidence of BC, compared to a <1% incidence of BC in BALB/c mice. BALB/c mice foster-nursed on C3H females have a high incidence of BC which suggested that the tumorigenic MMTV of C3H can be horizontally transmitted in the milk (Bittner, 1936). Conversely, when C3H mice are foster nursed on a BALB/c female the incidence of BC is significantly lower (22-55%), but not as low as low-incidence mouse strains. This latter observation underscores the importance of the horizontally-transmitted milk-born virus in the high incidence strains, but also indicates the substantial differences in tumorigenic potential among endogenous MMTV proviruses. Proviruses of various mice with high and low tumor incidence could be distinguished by differences in solution hybridization kinetics and restriction endonuclease digestion patterns (Cohen *et al. Cell*, 1979; Cohen and Varmus, 1979, 1980; Traina-Dorge and Cohen, 1983; Breznik *et al.* 1984). Using restriction enzymes that differentiate between hypomethylated and methylated DNA, proviruses of milk-borne MMTV were also shown to be hypomethylated, whereas most endogenous proviruses contain abundant 5-methylcytosine

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(Cohen, 1980; Breznik and Cohen, 1982; Breznik *et al.*, 1984). Because hypomethylation is associated with increased gene expression, this observation could explain the importance of horizontally-transmitted milk-borne MMTV in mouse strains with a high incidence of BC. Specific hypomethylation of an endogenous MMTV provirus was associated with expression of  
5 a 1.6 kb transcript of the lactating mammary gland of the BALB/c mouse (Traina-Dorge *et al.*, 1985).

In other studies, endogenous MMTV proviruses have been shown to segregate as stable genetic units during inbreeding and that certain of these endogenous proviruses are transcriptionally active (Cohen *et al.*, *Cell*, 1979; Cohen and Varmus, 1979, 1980; Traina *et al.*,  
10 1981; Traina-Dorge and Cohen, 1983; Traina-Dorge *et al.*, 1985; Varmus *et al.*, 1978). Recombinant inbred (RI) strains of mice have been used to define MMTV provirus composition and chromosomal locations (Traina *et al.*, 1981; Traina-Dorge and Cohen, 1983). RI strains were developed by crossing mice from two highly inbred lines, randomly mating the F2 generation brothers and sisters and maintaining each as separate lines. Many genetic loci were  
15 mapped to specific chromosomes in these strains. By analyzing the segregation patterns of various MMTV proviruses with these genetic markers by restriction endonuclease analysis and Southern blotting, it was possible to determine the chromosomal locations of a number of MMTV proviruses in these strains and to establish that these provirus segregate by the rule of Mendelian inheritance. These and other analyses defined 10 distinct MMTV units (proviruses)  
20 in these animals some full length and some truncated. The ratios of inheritance for most of the MMTV proviruses were consistent with simple single-gene inheritance, though three of the MMTV units demonstrated some variance. Most noticeable was the presence of a unit contributed by one parent that was present in 23 of the 26 RI strains analyzed. Specific proviral units were identified that were transcriptionally active and associated with increased tumor  
25 production (Traina-Dorge *et al.*, 1985). Importantly, cellular genes were identified that were significantly associated with virus expression showing the involvement of host genetics in disease progression (Traina-Dorge *et al.*, 1985).

Several lines of evidence indicate that the endogenous MMTV proviruses have integrated relatively recently in the germline of various strains of mice (Cohen and Varmus, 1979; Varmus  
30 *et al.*, 1978). If MMTV evolved from elements present in a progenitor of *Mus musculus* (the laboratory mouse), it would be expected that all individual mice would have similar MMTV

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proviruses. However, both laboratory mice and wild mice trapped at several locations having quite variable numbers and distributions of germline integration sites for their MMTV proviruses (Cohen and Varmus, 1979). The most striking finding among these results were the presence of some wild caught animals that contained no endogenous MMTV proviruses. The most likely interpretation of these results is that the endogenous MMTV proviruses arose by multiple independent integrations into the DNA of germinal cells after speciation of the genus *Mus*, rather than arising from genetic elements present in the evolutionary progenitors of mice.

### SUMMARY OF THE INVENTION

The present invention provides for recombinant DNA molecules derived from one or more mammary tumor viruses which are endogenous retroviruses with homology to the sequences of MMTV. Specifically, the sequences of the instant invention have at least 99% identity with all or a portion of SEQ ID NOs: 2, 3, 4, 5, 6, 7, 8, 21, 23, 25, 27, or 29 or at least 92% identity with SEQ ID NO:10.

Additionally, the present invention provides for the RNA molecules produced by transcription of the DNA described above. Furthermore it provides for the polypeptides resulting from the in-frame translation of RNA which has been transcribed from the MTV DNA of the instant invention. According to the present invention, the referenced DNA sequences may be derived from any suitable source, such sources may include, but are not limited to, human, cat, and rhesus macaque.

The present invention also provides for a recombinant DNA plasmid (a vector) which comprises mammary tumor virus (MTV) DNA the sequences of SEQ ID NOs: 2, 3, 4, 5, 6, 7, 8, 10, 21, 23, 25, 27, or 29, or sequences which have at least 99% identity thereto. That is said DNA sequence is incorporated in the vector.

In one embodiment of the present invention the vectors further comprise a heterologous promoter operably linked to the MTV sequence (*i.e.* joined in the proper reading frame so as to be capable of producing functional MTV RNA and/or protein *in vivo* and *in vitro*). In one aspect of this embodiment of the invention the vector, which comprises the MTV DNA, is capable of episomal replication or chromosomal integration in at least one of the following cell types: bacterial cells, yeast cells, insect cells, avian cells, and mammalian cells (this list of cell types is representative and should not be considered exhaustive). In another aspect, of this embodiment



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of the invention, the heterologous promoter provides for the expression of the MTV DNA sequence in one or more cell types. Cell types considered useful as part of this aspect of the invention include, but are not limited to the following: bacterial cells, yeast cells, insect cells, avian, cells, and mammalian cells.

5 According to another embodiment of the instant invention the MTV DNA sequences described above may be used to provide a method of detecting the presence of MTV DNA or RNA in a sample (of biological origin, such as serum, or otherwise).

Another embodiment of the instant invention provides for a method of determining whether a sample contains antibodies which recognize proteins derived from the MTV DNA  
10 sequences described above (e.g. polypeptide sequences derived from transcription and translation of SEQ ID NOs: 2, 3, 4, 5, 6, 7, 8, 10, 21, 23, 25, 27, or 29). As a corollary to this aspect, the instant invention also provides for antibodies which specifically detect one or more of the polypeptides of the instant invention.

Another embodiment of the invention provides for diagnostic kits useful for detecting  
15 DNA, RNA, or polypeptides, from a mammary tumor virus, in a biological or other type of sample.

Another embodiment of the instant invention provides for methods of attenuating or eliminating the activity of MTV in its host animal. Various aspects of this embodiment provide for pharmaceutical compositions comprising substances which disrupt the activity of the MTV  
20 reverse transcriptase, protease, or integrase enzymes. Other aspects of this embodiment provide for pharmaceutical compositions capable of eliciting an immune response in a host animal.

### BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by  
25 reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

Figure 1 shows the results of amplification of sequences related to MMTV from human breast cancer tissue. Panel A: DNA was extracted from human breast tumors (kindly provided by Michael Press, M.D., USC, Los Angeles or Derrick Beech M.D., TMC/UT Memphis) and  
30 PCR was performed using primers specific for the human MMTV *env*-related gene. PCR

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products were transferred to nitrocellulose by blotting and MMTV-related products were detected by hybridization to a 1.8 kb MMTV *env* probe. lane a: nonradioactive markers, not shown; lanes b-d, f-h, j: breast tumor DNA; lanes e and i: no DNA; lane k: water control; lane l positive control: MMTV *env* fragment cloned in pBluescript<sup>TM</sup>. Panel B: As a test for the integrity of the DNA from the clinical samples we amplified HERV-3 proviral DNA, a single copy human endogenous retrovirus using PCR conditions developed by Griffiths *et al.* (1997). Ethidium bromide detection (markers are visible in lane a). Same samples as Panel A except lane l: positive control, HERV3 *pol* fragment cloned in pBluescript<sup>TM</sup> (Stratagene). Visible bands were present in lanes c and g, but do not copy well.

Figure 2 shows an example of the amplification of sequences related to MMTV from the blood of healthy controls. Panel A: DNA was extracted from whole blood of healthy control subjects and PCR was performed using primers specific for the human MMTV *env*-related gene and PCR products were detected by Southern hybridization. lane a: markers; lanes b-k: DNA from whole blood of healthy controls; lane l: water control; lane m positive control: MMTV *env* fragment in pBluescript<sup>TM</sup> (Stratagene). Panel B: PCR amplification of HERV-3. Ethidium bromide detection (markers are visible in lane a). Same samples as Panel A except lane m: positive control, HERV3 *pol* fragment in pBluescript<sup>TM</sup>.

Figure 3 shows an example of the detection of HMTV mRNA by ribonuclease protection assay. RNA was extracted from three HMTV PCR positive breast cancer tumors or from yeast cells and hybridized to either 189 base probe specific for HMTV or a 245 base probe for  $\beta$ -actin. Samples were then digested with RNase A/T1. The fragments of the labeled probes protected by hybridized RNA were visualized and analyzed following separation on a denaturing polyacrylamide gel. Two of the three tumor samples gave protected fragments of the expected size with the HMTV probe (arrow) (lanes 1-3), whereas all three tumor RNAs gave protected fragments with the  $\beta$ -actin probe (lanes (5-7). As expected neither probe was specifically protected by yeast RNA (lanes 4, 8).

## DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for isolated DNA, RNA, and polypeptides derived from mammary tumor virus (MTV) which is a virus with high sequence homology to mouse mammary tumor virus. The present invention further provides for diagnostic methods of using

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these macromolecules. Additionally the present invention provides pharmaceutical reagent compositions and diagnostic kits comprising MTV DNA, RNA, and/or polypeptide, which can be used according to the disclosed methods.

Various embodiments of the instant invention provide for MTV nucleic acid  
5 corresponding to at least one of the following: DNA sequences with at least 99% identity with one of the following:

- a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or
- b) at least 99% identity with one or more of the following: at least 340 contiguous bases of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence represented by  
10 nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360 of SEQ ID NO:5; at least 130 contiguous nucleotides of SEQ ID NO:6, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:7, at  
15 least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170 contiguous nucleotides of SEQ ID NO:23, at least 100 contiguous nucleotides of the sequence represented by nucleotides 337-462 of SEQ ID NO:25, at least 300 contiguous nucleotides of the  
20 sequence represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or
- c) at least 92% identity with SEQ ID NO:10.

Preferably the sequences have greater than 99% identity with greater than 200, 250, 300, 350 or 400 nucleotides SEQ ID NO's: 2, 3, 4, 5, 6, 7, 8, 21, 23, 25, 27, or 29. In another  
25 preferred embodiment the sequences have greater than 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO:10. Even more preferably these nucleic acids are derived from human, cat, or rhesus macaque MTV's. Most preferably the DNA is identical with all or part of SEQ ID NO's: 2, 3, 4, 5, 6, 7, 8, 10, 21, 23, 25, 27, or 29.

In one aspect of this embodiment of the invention the MTV DNA sequences described  
30 above are incorporated in a vector. In various related aspects the invention the MTV sequences are under the transcriptional control of an heterologous promoter. The vectors contemplated as

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being useful according to the instant invention are capable of expressing the MTV DNA sequences in at least one of the following cell types: insect cells, bacterial cells, avian cells, yeast cells, or mammalian cells. Furthermore the vectors of this aspect of the invention are also capable of episomal replication and/or chromosomal integration in at least one of the cell types listed.

In another aspect of this embodiment the DNA sequences comprise one or more detection moieties. Detection moieties contemplated as being suitable for the instant invention include, but are not limited to fluorescent dyes, and radioactive isotopes of phosphorous, sulfur, oxygen, carbon, or hydrogen.

In yet another aspect of this embodiment of the invention the isolated DNA molecule is suspended or dissolved in a diluent compatible with the invention. Suitable diluents do not interfere with the use of the DNA according to the various embodiments of the instant invention. Diluents useful according to this embodiment of the invention are well known to those skilled in the art. They include but are not limited to buffered aqueous solutions. These may be buffered with any compound compatible with the present invention. Exemplary buffering agents include phosphate buffers and buffers comprising trishydroxyaminomethane (Tris). Such buffered aqueous solutions may further comprise any other compound, such as sodium chloride, which will not interfere with the operation of the instant invention. According to this aspect of the instant invention the MTV DNA may be present in any suitable concentration. Preferably the concentration is from about 0.1 ng/ $\mu$ l to 100  $\mu$ g/ $\mu$ l.

Other embodiments of the instant invention provide for RNA transcripts and/or polypeptides encoded by the nucleic acids described above. In various aspects of this embodiment these RNA transcripts or polypeptides are encoded by any of the DNA sequences described above. These RNA transcripts and polypeptides are homologues of RNA transcripts and polypeptides encoded by the MMTV *env*, *gag*, or *pol* genes. In one aspect of this embodiment the RNA transcripts are encoded by the DNA sequences described above. Such RNA transcripts have a sequence identical to the disclosed DNA sequence except that the RNA contains uridine instead of thymidine. In another preferred aspect of this embodiment the purified polypeptide corresponds in sequence with all or part of an *env*, *gag*, or *pro*, protein product from a human, cat, or rhesus macaque mammary tumor virus. It is preferred that the peptides be purified polypeptides. In a preferred aspect of the present invention the purified

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polypeptides are comprised of all or part of the sequence resulting from the in-frame transcription and translation of any of the MTV DNA sequences described above. In an even more preferred aspect of the present invention the polypeptides correspond in sequence to the product of an in-frame translation of SEQ ID NO's: 2, 3, 4, 5, 6, 7, 8, 10, 21, 23, 25, 27, or 29.

5 Most preferably the polypeptide sequences of the instant invention correspond to all or at least 80 amino acids of SEQ ID NO's 12, 13, 14, 15, 16, 17, 18, 20, 22, 24, 26, or 28. The term "derived from MTV DNA" is meant to convey that the RNA or peptide corresponds in sequence to the RNA or peptide produced respectively by the transcription or transcription and in-frame translation of MTV DNA.

10 By "purified polypeptides" it is meant that the majority (greater than 50%) of the polypeptides in the sample are the MTV polypeptides. Preferably, the MTV polypeptides constitute greater than 70% of the polypeptide in the purified polypeptide sample. More preferably the MTV polypeptide constitutes greater than 90% of the polypeptide in the sample. Even more preferably the MTV polypeptide constitutes greater than 95% of the polypeptide in  
15 the sample. Additionally, the term "purified polypeptide" indicates that the sample does not contain substances which interfere with the operation of the instant invention. Purified polypeptides obtained in accordance with the current invention may be from any suitable source which is compatible with the instant invention.

Another embodiment of the instant invention provides for an antibody against an MTV  
20 polypeptide as described above. Antibodies according to this aspect of the invention are generated using one or more of the polypeptides of the instant invention as the antigenic agent. Such antibodies may be either monoclonal or polyclonal in nature. Preferably the antibodies are monoclonal. Once the MTV has been provided the antibodies of this aspect of the invention may be prepared according to methods well known to those skilled in the art. For example,  
25 polyclonal antibodies are commonly produced by injecting the antigenic agent (in the presence of an immune response enhancing agent such as complete Freund's adjuvant) into an animal such as a sheep, bovine, equine, goat, or rabbit.

Monoclonal antibodies according to this aspect of the present invention can be prepared by hybridoma fusion techniques or by techniques that utilize Epstein Barr Virus (EBV) -  
30 immortalization technologies (to produce human mAbs), such as are well known by those of skill in the art, modified as described herein. In the method of the invention, these techniques involve

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the injection of an immunogen, in this case, purified MTV polypeptide so as to elicit a desired immune response in that animal (i.e., production of antibodies). The experimental animal, (e.g., a mouse) is given repeated injections (boosts) of the same immortalized cell line. In a final step, the animal is given an injection of primary cells of the chosen cell type.

5 In the illustrative example herein for the production of agonist monoclonal antibodies to megakaryocytic cells, a CMK cell preparation and a CMS cell preparation were used as the first immunogens; however, other immortalized megakaryocytic cells, such as Mo7e or DAMI cells, could have been used. Other monoclonal antibodies analogous to the agonist antibody of the invention BAH-1, which specifically recognizes the C-Mpl receptor, can be generated using  
10 membrane bound c-Mpl receptor protein as the immunogen. To generate agonist monoclonal antibodies against other cell types, other cells of hemopoietic lineage are chosen, e.g., stem cells, B cells or T cells. In the first immunization step stem cells can be represented, e.g., by the immortalized cell line CTS; B cells by the immortalized cell lines ARH-77, SB or Nal-6; and T cells by the immortalized cell lines Jurkat or H9.

15 After a sufficient time, the animal is sacrificed and somatic antibody-producing cells may be derived from the lymph nodes, spleens and peripheral blood of primed animals. Spleen cells are preferred. Mouse lymphocytes give a higher percentage of stable fusions with the mouse myelomas described below. The use of rat, rabbit, frog, sheep and other mammalian somatic cells is also possible. The spleen cell chromosomes encoding desired immunoglobulins are  
20 immortalized by fusing the spleen cells with myeloma cells, generally in the presence of a fusing agent such as polyethylene glycol (PEG). Any of a number of myeloma cell lines may be used as a fusion partner according to standard techniques; for example, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110-2209.

25 The resulting cells, which include the desired hybridomas, are then grown in a selective medium, such as HAT medium, in which unfused parental myeloma or lymphocyte cells eventually die. Only the hybridoma cells survive and can be grown under limiting dilution conditions to obtain isolated clones. The supernatants of the hybridomas are screened for the presence of antibody of the desired specificity, e.g., by immunoassay techniques such as those  
30 described herein, using the antigen that has been used for immunization. Positive clones can then be subcloned under limiting dilution conditions and the monoclonal antibody produced can be

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isolated. Various conventional methods exist for isolation and purification of the monoclonal antibodies so as to free them from other proteins and other contaminants. Commonly used methods for purifying monoclonal antibodies include ammonium sulfate precipitation, ion exchange chromatography, and affinity chromatography. Hybridomas produced according to these methods can be propagated in vitro or in vivo (in ascites fluid) using techniques known in the art (see, generally, Harlow et al., Antibodies. A Laboratory Manual, Cold Spring Harbor Laboratory, pp. 1-726, 1988).

Other embodiments of the instant invention provide methods for detecting DNA, RNA, and/or proteins from MTV in biological and/or other types of samples.

One aspect of this embodiment of the invention provides a method for the detection of MTV DNA in a sample which comprises the following steps:

- i) obtaining an sample suspected of containing one or more of the MTV DNA sequences described above;
- ii) carrying out a polymerase chain reaction (PCR) to amplify a DNA sequence as defined in step i); and,
- iii) determining the sequence of, or otherwise characterizing, the amplicons (the PCR amplified DNA) produced in step ii) to determine whether or not the DNA sequence as defined in step i) is present in the sample.

Another aspect of this embodiment provides a method for the detection of MTV RNA in a sample which comprises the following steps:

- i) obtaining an sample suspected of containing RNA which encoded by one or more of the MTV DNA sequences described above;
- iii) carrying out an RNase protection assay (RPA); and,
- iii) analyzing the RPA results to determine whether RNA as defined in step i) is present in the sample and optionally quantitating said RNA.

It will be recognized by those of ordinary skill that the selection of the parameters necessary for optimizing the steps of these methods are well within the abilities of the ordinarily skilled artisan. These parameters, for example PCR conditions (e.g. selection of annealing temperature, extension times, and primers) and DNA sequencing method, are routinely determined in labs where such molecular biological techniques are employed.

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Another aspect of this embodiment of the invention provides for a method for analyzing a sample in order to determine whether the sample contains antibodies which recognize MTV polypeptides. This method comprises the steps of:

- i) obtaining a sample suspected of containing antibodies specific for mammary tumor viral antibodies;
- ii) obtaining at least one purified MTV polypeptide;
- iii) performing western immunoblot analysis using the sample of step i) and the polypeptide of step ii); and,
- iv) analyzing the results of step iii) to determine whether or not antibodies which specifically interact with the peptide of step ii) are present in the sample.

Another aspect of this embodiment of the invention provides for a method for analyzing a cell culture or a tissue sample by one or more immunohistological methods in order to determine whether the sample contains MTV proteins. This method comprises the steps of:

- i) obtaining a sample suspected of containing mammary tumor viral proteins;
- ii) preparing the sample of step i) for immunochemical analysis;
- iii) incubating the sample of step ii) with one or a combination of two or more monoclonal or polyclonal antibodies specific for MTV polypeptides encoded by one or more of the MTV DNA sequences described above, wherein the said antibodies optionally have a moiety which allows for their specific detection;
- iv) washing the samples to remove antibody which is not specifically bound;
- v) processing the samples as appropriate for the selected detection method; and,
- vi) analyzing the results of step v.) to determine whether or not MTV proteins are present in the sample.

It is envisioned that the immunochemical analysis may include, but is not limited to analysis by western blotting or enzyme-linked immunosorbant assay. It will be recognized that the selection of the parameters necessary for optimizing the performance of these methods are within the abilities of the ordinarily skilled artisan. Analysis may be performed using either direct immunochemistry (if the antibodies have been labeled with a detectable moiety) or by indirect immunochemistry.

Other embodiments of the current invention provide for pharmaceutical compositions comprising MTV DNA, RNA and/or proteins described *supra*. These compositions may further



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comprise a pharmaceutically acceptable excipient, carrier, or diluent and do not contain any biologically harmful substances. The pharmaceutical compositions of the present invention may be formulated by one having ordinary skill in the art. Suitable pharmaceutical formulations are described in *Remington's Pharmaceutical Sciences* which is a standard reference text in the field  
5 which is here incorporated by reference.

The pharmaceutical compositions may further comprise coloring or stabilizing agents, osmotic agents, antibacterial agents, or any other substances as long as such substances do not interfere with the function of the composition. The pharmaceutical compositions of the invention, can, for example, be formulated as a solution, suspension, or emulsion in association  
10 with a pharmaceutically acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human albumen. Liposomes may also be used. The vehicle may contain additives that maintain isotonicity (e.g., sodium chloride or mannitol) and chemical stability (e.g., buffers and preservatives). It should be appreciated that endotoxin contamination should be kept at a safe level, for example, less than 0.5 ng/mg protein.  
15 Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by the United States Food and Drug Administration Office of Biological Standards. The formulations may be sterilized by commonly used techniques such as filtration.

The phrase "pharmaceutically acceptable" refers to substances and compositions which  
20 do not produce an adverse, allergic, or otherwise untoward reaction when administered to an animal, or a human, as appropriate. A substance which caused produced any of these adverse effects would be classified as "biologically harmful" within the scope of the present invention. Pharmaceutically acceptable substances and compositions include, but are not limited to solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption  
25 delaying agents. Except where incompatible with the invention the use of any conventional ingredient is contemplated. Furthermore, supplementary active ingredients which serve some other pharmacologically expedient purpose can also be incorporated into the instant compositions.

The methods and compositions described above are contemplated to be of great benefit in  
30 helping to determine whether or not MTV or MTV viral components are present in a person's tissues. This knowledge is would be of aid in predicting a patient's susceptibility to cancer

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etiologically derived from MTV. The methods provide a means for determining a patient's viral load (the amount of virus present in the person's tissues). In this respect it is envisioned that tremendous benefit would be achieved by adapting the methods herein described to the widespread screening of the general population, and in particular to all mature human females.

5 Other compositions contemplated as part of various embodiments of the instant invention provide a means for stabilizing or reducing the amount of virus present in a human or other animal.

The various methods described above are readily adaptable to preparing diagnostic kits for detecting the presence of MTV DNA, RNA, and/or polypeptides. Consequently, for the  
10 clinical practice of the invention, yet other embodiments of the instant invention, which provide for diagnostic kits, are contemplated. Such kits are useful for the qualitative and quantitative analysis of a sample in order to detect, and perhaps determine the quantity of, MTV DNA, RNA, and/or polypeptide present therein. In one aspect of this embodiment of the invention the kit includes, as part of its components, one or more recombinant DNA molecules comprising one or  
15 more of the MTV DNA sequences described above. Also according to this aspect of the invention the kit may comprise, in addition to (or in alternative to) the recombinant MTV DNA, one or more synthetic oligonucleotide primer pairs useful for the PCR amplification of these MTV DNA sequences.

Another aspect of this embodiment of the invention provides for a kit for detecting  
20 antibodies which specifically recognize mammary tumor virus proteins. As part of its components this kit includes a reagent comprising one or more polypeptides encoded by the MTV DNA sequences described above.

Also contemplated are kits useful for detecting the presence of MTV proteins by immunocytochemistry which comprise one or more antibodies (either monoclonal or polyclonal)  
25 which are specific for at least one MTV polypeptide. Optionally, these antibodies may be modified so as to comprise a detection moiety (*e.g.*, a fluorescent dye or a radioactive isotope, such as <sup>35</sup>S).

Kits according to these embodiments of the invention may comprise packages, each containing one or more of the various reagents (typically in concentrated form) which are  
30 required to perform the respective diagnostic tests. The kits according to these embodiments of the invention are contemplated to be useful for detecting and/or quantifying MTV DNA, RNA,

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and/or protein in biological (or other types of) samples. Such samples may be selected from, but are not limited to the following: blood plasma or serum, whole blood, urine, sputum, colonic effluent, cerebrospinal fluid, lymphatic fluid, bone marrow, tissue samples (such as from a surgical biopsy), or any other sample suspected of containing these biological molecules.

5       The kits also may further include one or more fiduciary results. As used herein a "fiduciary result" refers to a reference standard against which a test outcome is compared to gauge the results in terms of quality and/or quantity. A "fiduciary series" is a plurality of such references that represent points along a qualitative or a quantitative scale. Preferable in this regard, are kits that include a fiduciary series for interpreting results. The fiduciary may be in  
10   the form of one or more photographs or may be depicted in other ways, including written descriptions.

Thus fiduciaries may be developed as part of the kits, in accordance with this aspect of the invention, to guide interpretation of results. In this regard, a RNA or polypeptide concentration from a biological sample may be characterized in accordance with the foregoing.  
15   Sample may be taken from representative cross-section of patients at various stages of disease (including asymptomatic or essentially disease free) to prepare a fiduciary series. Characterization in this regard may benefit from hindsight, by following the actual course of neoplastic progression in patients as they undergo diagnosis, treatment, and follow up thereafter. The determination of viral load (as determined by the abundance of MTV DNA, RNA, or  
20   polypeptide), as set out above, for a variety of patients of known breast cancer status and eventual outcome, and the subsequent correlation of these values is of incalculable prognostic value. This will help provide the patient with a more accurate prognosis, and it will also aid the patient and oncologist in determining the best course of therapeutic treatment, when such treatment is necessary.

25       Another embodiment of the instant invention provides for a composition which induces an immunological response against MTV in an animal. A specifically contemplated aspect of this invention is a composition which induces an immunological response to human mammary tumor viral protein in humans (see Example 7). It is expected that such induced immunity may prevent breast cancer in individuals that carry endogenous MTV, by blocking the spread of the  
30   virus to hormonally sensitive tissues.

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Methods for producing an immune response to viral DNA and/or RNA in animals are also known, see for example U.S. patent numbers 5,990,091 and 6,004,799 which are herein incorporated by reference. Thus compositions which elicit an immune response to MTV DNA and/or RNA are also contemplated as aspect of this embodiment of the invention.

5 Other embodiments provide for therapeutic compositions which decrease the activity and presence of human mammary tumor virus. A common feature of all retroviruses is the presence of three enzymes involved in various stages of the viral replication cycle, the reverse transcriptase (RT), protease (PR), and integrase (IN). Various pharmaceuticals have been developed which target the RT and PR of human immunodeficiency virus (HIV), a retrovirus  
10 distantly related to MTV. Furthermore, chemicals which selectively inhibit HIV IN have been identified and prototype drugs targeting this enzyme are under development (Hong *et al.*, 1998; Mathe, 1999; Robinson, 1998; Singh *et al.*, 2000). According to one aspect of the instant invention a pharmaceutical composition comprising one or a mixture of two or more retroviral inhibitors in an amount effective to inhibit the activity or the spread of MTV's of the instant  
15 invention is provided.

Identification of the drug or drugs for use in the pharmaceutical compositions of this embodiment can be made using techniques known to those skilled in the art. For examples of protease and reverse transcriptase inhibitors and methods of determining the efficacy of such drugs as retroviral inhibitors see U.S. Patent Nos. 5,858,738, 6,017,928, and 6,046,228, which  
20 are herein incorporated by reference. The various compositions of this embodiment of the invention may comprise, for example, currently known HIV integrase, protease, or reverse transcriptase inhibitors; alternatively, such inhibitors can be modified so as to increase their specificity for MTV's. Likewise, other classes of HIV inhibitors, such as peptides which are analogs of the transmembrane glycoprotein, can serve as pro-drugs to develop specific drugs  
25 which decrease activity and presence of MTV in humans and other species. These compositions are contemplated as being useful to inhibit the activity and spread of MTV in humans and other species which carry these viruses as endogenous genetic elements. Such drugs could be used to treat breast cancer patients afflicted with MTV and would be expected to ameliorate the severity and to reduce the recurrence of disease. In addition, such drugs could be used prophylactically to  
30 prevent the occurrence of individuals at high risk for developing the disease.

## EXAMPLES

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

### EXAMPLE 1: MMTV-related sequences in humans

Sequences which are highly similar (>95%) to the MMTV *env* gene were amplified by PCR from human DNA samples, including subsets of both BC (breast cancer) tissue and non-BC tissues. The MMTV-related sequences were found by PCR and a sensitive blotting technique not only in breast tumors (see Figure 1), but also in the blood of a subset of healthy controls (see Figure 2), and systemic lupus erythematosus (SLE) patients without breast cancer. Our results differ from those of Wang and coworkers (1995) who, with few exceptions, were able to detect MMTV-like sequences only in breast tumors. The sequences from human DNA were distinct from the MMTV sequences used as controls in these PCR reactions indicating that our results are not simply due to contamination. A ribonuclease protection assay was used to confirm these results using a non-PCR based technique to determine that the majority of the PCR positive BC tissues, but none of the PCR negative tissues, expressed this sequence at the mRNA level. Many of the products from these PCR reactions have been sequenced. Analysis of these sequences provides further strong evidence that PCR contamination is an unlikely explanation for the observed results. MMTV *env*-like sequences from different individuals derived in the same PCR run were distinct from each other. This result indicates the lack of an ubiquitous PCR contaminant that would have produced a more consistent sequence that should have been identical (or nearly so) in the various reaction tubes. Furthermore DNA of individual subjects produced internally consistent MMTV *env*-like sequences from PCR run to PCR run. The variations within the MMTV-related sequences from a given patient may represent a low number of Taq errors, but are also suggestive of variations expected of a replicating retrovirus (reverse transcriptase errors).

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**Example 2: RNase Protection Assay determination of MTV RNA levels**

Ribonuclease protection assay (RPA) is a quantitative assay which is frequently used by those of ordinary skill in the art to determine the of levels of specific RNA species without PCR amplification. Briefly, it is performed as follows for the transcripts of the present invention: a probe of uniform length is synthesized by *in vitro* transcription of a cloned template and labeled to high specific activity. <sup>35</sup>S-labeled riboprobes were prepared from plasmids containing the cloned MTV fragments in the 150 to 400 bp size range. The probe was then hybridized to test RNA, and RNA fragments that remain unhybridized are not protected from digestion by RNase A/T1. The fragments of the labeled riboprobe protected by hybridized RNA were visualized and analyzed following separation on a denaturing polyacrylamide gel. Levels of MTV-related mRNA are compared in each sample to levels of mRNA produced by the  $\beta$ -actin gene, a "housekeeping" gene to ensure integrity of the RNA sample (*i.e.*, that the RNA has not been degraded). RPA detected RNA in 2 out of 3 PCR positive breast tumors (see Figure 3). RPA is 10-15 times more sensitive than "northern" analysis for detection of rare messenger RNA. RPA analysis is performed directly on total RNA, without any prior manipulation, which can introduce errors in the quantitative analysis.

**Example 3: RT-PCR determination of MTV RNA levels**

MTV mRNAs may also be detected using real time RT-PCR (reverse transcriptase linked polymerase chain reaction). For example the I-Cycler (Biorad), with a fluoroscopic detection facility is capable of determining the real time kinetics of the PCR amplification product by quantifying the PCR product in the log-linear phase of the PCR reaction. Using this method, a real-time thermocycler can reliably compare miniscule amounts of known nucleotide template in a reproducible fashion.

**Example 4: Detection of anti-MTV antibodies in blood by Western blot analysis**

The InsectSelect<sup>TM</sup> System (Invitrogen) which allows stable production of recombinant proteins in insect cells in a manner similar to well-characterized baculovirus expression systems may be used to generate recombinant MTV proteins. This is a virus-free system which allows creation of stable cell lines that continuously produce high-quality protein. Stable cell lines generated in about 9 days may be used for continuous, long-term production of recombinant proteins. The InsectSelect<sup>TM</sup> expression system is based on a plasmid vector which carries an

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antibiotic resistance gene for selecting stably expressing insect cell lines. This expression vector uses the immediate early promoter, OpIE2, from the Douglas Fir Tussock moth OpMNVP baculovirus for gene expression. OpIE2 is a strong transcriptional promoter in lepidopteran (Sf9, 5121, High Five<sup>TM</sup> (Invitrogen)) as well as mosquito and dipteran cell lines. The expression  
5 vector pIZ/V5-His (Invitrogen) has a multiple cloning site and several features that simplify production and analysis of recombinant proteins in insect cells. It includes a Zeocin-resistance gene that allows for rapid selection of stably transfected cells, a C-terminal tag encoding the V5 epitope, and a C-terminal polyhistidine (6xHis) sequence. These latter features facilitate rapid protein detection with anti-V5 antibodies (Invitrogen) and protein purification with resins that  
10 bind the polyhistidine. Alternative eukaryotic expression systems for MMTV related proteins are an *in vitro* rabbit reticulocyte lysate system (Promega) with *in vitro* transcribed and capped mRNA or the Sindbis virus Expression system (Invitrogen).

MMTV-related genes may also be cloned into a pGEX vector (Pharmacia) and expressed in bacteria to make recombinant fusion protein with glutathione S-transferase (GST) protein for  
15 immunoblot studies. This system has several advantages compared to other methodologies of protein expression, not the least of which is the ease of production, isolation, and purification of the recombinant protein. Fusion proteins generated with the GST protein typically remain soluble permitting the recovery from cell lysates. Denaturing conditions are not required during purification and therefore the antigenic and enzyme properties of the protein are often  
20 maintained. Moreover, the GST fusion protein may be efficiently and rapidly purified by immobilizing the GST on glutathione coated beads or columns and eluted with reduced glutathione.

To perform a western immunoblot, MTV proteins are dissolved in lysis buffer (0.25 M Tris Base, pH 6.8, containing 4% sodium dodecyl sulfate, 10% dithiothreitol, 20% glycerol, and  
25 0.01% w/v bromophenol blue), heated to 100°C for 3 min and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% polyacrylamide gel. These proteins may then be transferred electrophoretically to a nitrocellulose membrane (Protran<sup>TM</sup>, Schleicher & Schuell) in Tris-glycine (pH 8.3) buffer with 20% methanol. The blots may be incubated overnight in blocking buffer (0.02 M Tris, 0.1 M NaCl, heat inactivated goat serum,  
30 0.01% thimerosal, and 5% nonfat dry milk) with serum or plasma from the human or animal being tested (1:100 dilution or optimal dilution). Incubation with secondary antibodies,

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biotinylated anti-human (or other species) IgG goat antibodies diluted 1:500 or 1:1,000 in blocking buffer, and with avidin-horseradish peroxidase may be performed at room temperature for 2 hours. The immunoblots may be developed with 7.8 mM 4-chloro-1-naphthol and 0.03% hydrogen peroxide. Bands corresponding to MTV proteins are quantitated by scanning and processed with image analysis software (NIH Image).

**Example 5: Detection of anti-MTV antibodies by enzyme linked immunoassay**

Antibodies which specifically bind to MTV protein(s) may be detected using an enzyme-linked immunoassay with MTV protein or proteins as the target for the antibodies. In this technique MTV proteins produced in an expression system, such as the baculovirus/insect cell clutter system or purified from virus preparations may be bound to the bottom of wells in a multiwell plastic microtiter plate. Serum or plasma from humans or other species is diluted to an empirically determined optimum dilution and incubated from 1 hour to overnight at about 25 °C (room temperature). The wells are then washed three times in a saline solution containing Tween® 20 (Aldrich) or NP-40 (currently available as Igepal™ CA-630, Sigma) detergents using an automated plate washer. Antibodies bound to the MTV proteins may then be detected by reacting the wells sequentially with buffers containing biotinylated goat anti-human immunoglobulin, followed by avidin coupled to horseradish peroxidase, and finally 3,3',5,5'-tetramethylbenzidine a substrate for horseradish peroxidase which produces a colored reaction product. Between each step the well is typically washed three times in a saline solution containing Tween® 20 or NP-40 detergents using an automated plate washer. The colored reaction produced in each well is quantitated using a spectrophotometer plate reader. The amount of colored reaction product is proportional to the amount of antibodies to MTV present in the original sample.

**Example 6: Detection of MTV retroviral proteins in situ using immunohistochemistry**

MTV retroviral proteins may be detected in tissue samples by immunohistochemical assays. In this technique MTV proteins produced in an expression system, such as the baculovirus/insect cell clutter system or purified from virus preparations may be used to generate monoclonal or polyclonal antibodies by methods well known to those skilled in the art. These antibodies may then be labeled with a detection moiety such as biotin, fluorescein, horseradish peroxidase or other labels used for this purpose. The labeled antibody preparations are then



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diluted to an empirically determined optimum dilution and incubated with fixed or frozen thin sections or cell preparations from samples to be tested for the presence of MTV proteins. The samples are processed, as dictated by the type of label used, and the binding of the antibody to MTV retroviral proteins may be detected by microscopy, autoradiography, flow cytometry, etc.,  
5 again, as dictated by the identity of the labeling moiety.

**Example 7: Preparation of a pharmaceutical composition capable of eliciting an immunological response, against human mammary tumor virus, in humans.**

A pharmaceutical composition to be used to induce an immunological response in humans or animals to MTV protein may be developed as follows. MTV proteins produced in an  
10 expression system, such as the baculovirus/insect cell culture system described above or purified from virus preparations may be extensively purified using column chromatography and/or any other methodologies commonly employed by skilled artisans. The purified MTV proteins may then be mixed or emulsified with a suitable adjuvant preparation (currently alum is the only adjuvant approved for use in humans) to produce a vaccine. This immunogenic composition  
15 may then be injected subcutaneously or intradermally into the target animal.

All of the composition and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the  
20 compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are  
25 deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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**CLAIMS:**

1. An isolated DNA molecule comprising a DNA sequence that has:
  - a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or
  - 5 b) at least 99% identity with one or more of the following: at least 340 contiguous bases of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360 of SEQ ID NO:5, at
  - 10 least 130 contiguous nucleotides of SEQ ID NO:6, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:7, at least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170 contiguous nucleotides of SEQ ID
  - 15 NO:23, at least 100 contiguous nucleotides of the sequence represented by nucleotides 337-462 of SEQ ID NO:25, at least 300 contiguous nucleotides of the sequence represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or
  - c) at least 92% identity with SEQ ID NO:10.
- 20 2. The isolated DNA molecule of claim 1 which comprises SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, 10, 21, 23, 25, 27, or 29.
3. The isolated DNA molecule of claim 1 wherein the DNA sequence is derived from human, rhesus macaque, or cat.
4. The isolated DNA molecule of claim 1, further comprising a detection moiety.
- 25 5. The isolated DNA molecule of claim 1, which is suspended or dissolved in a diluent.
6. The isolated DNA molecule of claim 5 wherein the diluent is a buffered aqueous solution.

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7. The isolated DNA molecule of claim 5 wherein the DNA is present at a concentration of from about 0.1 ng/ $\mu$ l to 100  $\mu$ g/ $\mu$ l.

8. A purified polypeptide encoded by a DNA molecule comprising a DNA sequence that has:

5 a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or

b) at least 99% identity with one or more of the following: at least 340 contiguous bases of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360 of SEQ ID NO:5, at least 130 contiguous nucleotides of SEQ ID NO:6, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:7, at least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170 contiguous nucleotides of SEQ ID NO:23, at least 100 contiguous nucleotides of the sequence represented by nucleotides 337-462 of SEQ ID NO:25, at least 300 contiguous nucleotides of the sequence represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or

20 c) at least 92% identity with SEQ ID NO:10.

9. The purified polypeptide of claim 8 which is SEQ ID NO: 12, 13, 14, 15, 16, 17, 18, 20, 22, 24, 26, 28, or 30.

10. The purified polypeptide of claim 8 which is present in a pharmaceutical composition.

11. An antibody which recognizes a polypeptide encoded by a DNA molecule comprising a DNA sequence that has:

25 a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or

b) at least 99% identity with one or more of the following: at least 340 contiguous bases of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the

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nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360 of SEQ ID NO:5, at least 130 contiguous nucleotides of SEQ ID NO:6, at least 140 contiguous  
 5 nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:7, at least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170 contiguous nucleotides of SEQ ID NO:23, at least 100 contiguous nucleotides of the sequence represented by  
 10 nucleotides 337-462 of SEQ ID NO:25, at least 300 contiguous nucleotides of the sequence represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or

c) at least 92% identity with SEQ ID NO:10;  
 wherein said antibody was generated using said polypeptide.

12. The antibody of claim 11 which is a monoclonal antibody.

13. An RNA corresponding to a DNA molecule a DNA molecule comprising a DNA sequence that has:

a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or

b) at least 99% identity with one or more of the following: at least 340 contiguous bases  
 20 of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360 of SEQ ID NO:5, at least 130 contiguous nucleotides of SEQ ID NO:6, at least 140 contiguous  
 25 nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:7, at least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170 contiguous nucleotides of SEQ ID NO:23, at least 100 contiguous nucleotides of the sequence represented by  
 30 nucleotides 337-462 of SEQ ID NO:25, at least 300 contiguous nucleotides of the



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sequence represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or

c) at least 92% identity with SEQ ID NO:10.

14. The isolated DNA molecule of claim 1 which is incorporated in a vector;  
5 wherein said DNA sequence is under the transcriptional control of a heterologous promoter.

15. The vector of claim 14 which is capable of expressing the DNA sequence in at least one of the following cell types: insect cells, bacterial cells, avian cells, yeast cells, or mammalian cells.

10 16. The vector of claim 14 wherein said vector is capable of episomal replication or chromosomal integration in at least one of the following cell types: insect cells, bacterial cells, avian cells, yeast cells, or mammalian cells.

17. A method for the detecting the presence of a mammary tumor viral DNA comprising the steps of:

15 i) obtaining a biological sample suspected of containing DNA which comprises a sequence molecule a DNA molecule comprising a DNA sequence that has:

a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or

b) at least 99% identity with one or more of the following: at least 340 contiguous bases of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence  
20 represented by nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360 of SEQ ID NO:5, at least 130 contiguous nucleotides of SEQ ID NO:6, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-380 of  
25 SEQ ID NO:7, at least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170 contiguous nucleotides of SEQ ID NO:23, at least 100 contiguous nucleotides of the sequence represented by nucleotides 337-462 of SEQ ID NO:25, at least 300

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contiguous nucleotides of the sequence represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or

- c) at least 92% identity with SEQ ID NO:10;
  - ii) carrying out a polymerase chain reaction to amplify the suspected DNA sequence as defined in step i); and,
  - iii) determining the sequence of, or otherwise characterizing, the amplicons produced in step ii) to determine whether or not the DNA sequence as defined in step i) is present in the sample.
18. The method of claim 17 wherein the biological sample is obtained from human, rhesus macaque, or cat.
19. A method of detecting the presence of antibodies which recognize one or more mammary tumor virus polypeptides, the method comprising the steps of:
- i) obtaining a sample suspected of containing antibodies specific for mammary tumor viral antibodies;
  - ii) obtaining at least one purified polypeptide encoded by a DNA molecule comprising a DNA sequence that has:
    - a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or
    - b) at least 99% identity with one or more of the following: at least 340 contiguous bases of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360 of SEQ ID NO:5, at least 130 contiguous nucleotides of SEQ ID NO:6, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:7, at least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170 contiguous nucleotides of SEQ ID NO:23, at least 100 contiguous nucleotides of the sequence represented by nucleotides 337-462 of SEQ ID NO:25, at least 300 contiguous nucleotides of the sequence

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represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or

c) at least 92% identity with SEQ ID NO:10;

iii) performing an immunochemical analysis using the sample of step i) and the polypeptide of step ii); and,

iv) analyzing the results of step iii) to determine whether or not antibodies which specifically interact with the peptide of step ii) are present in the sample.

20. The method of claim 19 wherein the immunochemical analysis of step iii) is a Western blot analysis.

10 21. The method of claim 19 wherein the immunochemical analysis of step iii) is enzyme-linked immunosorbant assay (ELIZA) analysis.

22. The method of claim 19 wherein one or more of the polypeptides are derived from the *env*, or *gag*, gene and the antibodies are specific for one or more of the following polypeptides SEQ ID NO: 12, 13, 14, 15, 16, 17, 18, 20, 22, 24, 26, 28, or 30.

15 23. The method of claim 19 wherein the sample of step ii) comprises antibodies derived from a human, rhesus macaque, or cat.

24. A diagnostic kit for detecting DNA or RNA from a mammary tumor virus, said kit comprising a reagent of a DNA molecule that comprising a DNA sequence that has:

a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or

20 b) at least 99% identity with one or more of the following: at least 340 contiguous bases of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360 of SEQ ID NO:5, at  
25 at least 130 contiguous nucleotides of SEQ ID NO:6, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:7, at least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170 contiguous nucleotides of SEQ ID  
30 NO:23, at least 100 contiguous nucleotides of the sequence represented by

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nucleotides 337-462 of SEQ ID NO:25, at least 300 contiguous nucleotides of the sequence represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or

c) at least 92% identity with SEQ ID NO:10.

5 25. A diagnostic kit for detecting antibodies to mammary tumor virus, where said kit comprises a reagent comprises one or both of the following:

i) one or more polypeptides encoded by a DNA molecule comprising a DNA sequence that has:

a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or

10 b) at least 99% identity with one or more of the following: at least 340 contiguous bases of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360 of SEQ ID NO:5, at least 130 contiguous nucleotides of SEQ ID NO:6, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:7, at least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170 contiguous nucleotides of SEQ ID NO:23, at least 100 contiguous nucleotides of the sequence represented by nucleotides 337-462 of SEQ ID NO:25, at least 300 contiguous nucleotides of the sequence represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or

20 c) at least 92% identity with SEQ ID NO:10; or

25 ii) an antibody specific for a polypeptide according to step i).

26. A method for the detection of MTV RNA in a sample which comprises the following steps:

i) obtaining an sample suspected of containing RNA which encoded by one or more DNA sequences that have:

30 a) at least 99% identity with SEQ ID NO: 2, 3, 4, 5, 7, 8, 21, 25, or 27; or

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- b) at least 99% identity with one or more of the following: at least 340 contiguous bases of SEQ ID NO:2, at least 150 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:3, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-400 of SEQ ID NO:4, at least 130 contiguous nucleotides of the sequence represented by nucleotides 1-360 of SEQ ID NO:5, at least 130 contiguous nucleotides of SEQ ID NO:6, at least 140 contiguous nucleotides of the sequence represented by nucleotides 1-380 of SEQ ID NO:7, at least 210 contiguous nucleotides of the sequence represented by nucleotides 20-462 of SEQ ID NO:8, at least 160 contiguous nucleotides of the sequence represented by nucleotides 97-462 of SEQ ID NO:21, at least 170 contiguous nucleotides of SEQ ID NO:23, at least 100 contiguous nucleotides of the sequence represented by nucleotides 337-462 of SEQ ID NO:25, at least 300 contiguous nucleotides of the sequence represented by nucleotides 85-462 of SEQ ID NO:27, or at least 200 contiguous nucleotides of SEQ ID NO:29; or
- c) at least 92% identity with SEQ ID NO:10;;
- iv) carrying out an RNase protection assay (RPA); and,
- iii) analyzing the RPA results to determine whether RNA as defined in step i) is present in the sample and optionally quantitating said RNA.

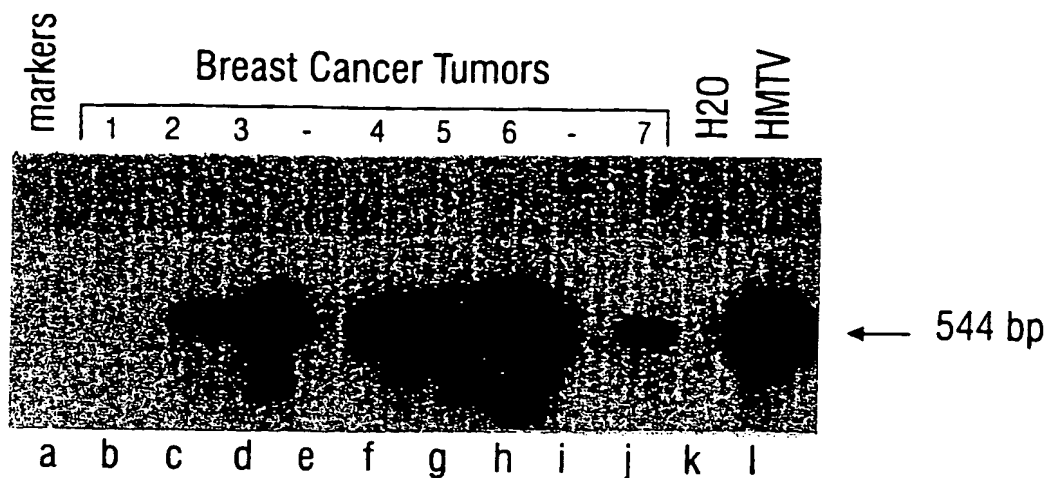


FIG. 1A

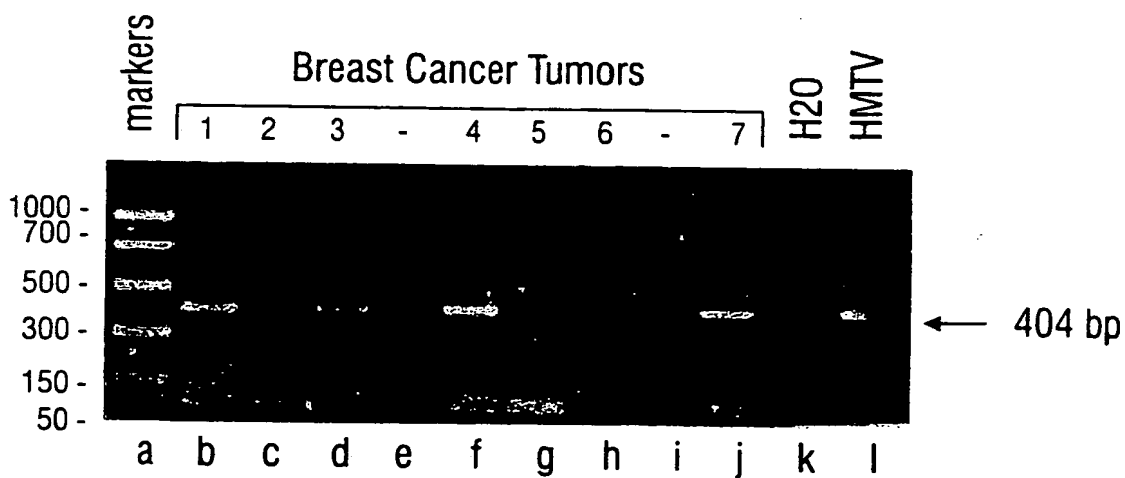


FIG. 1B

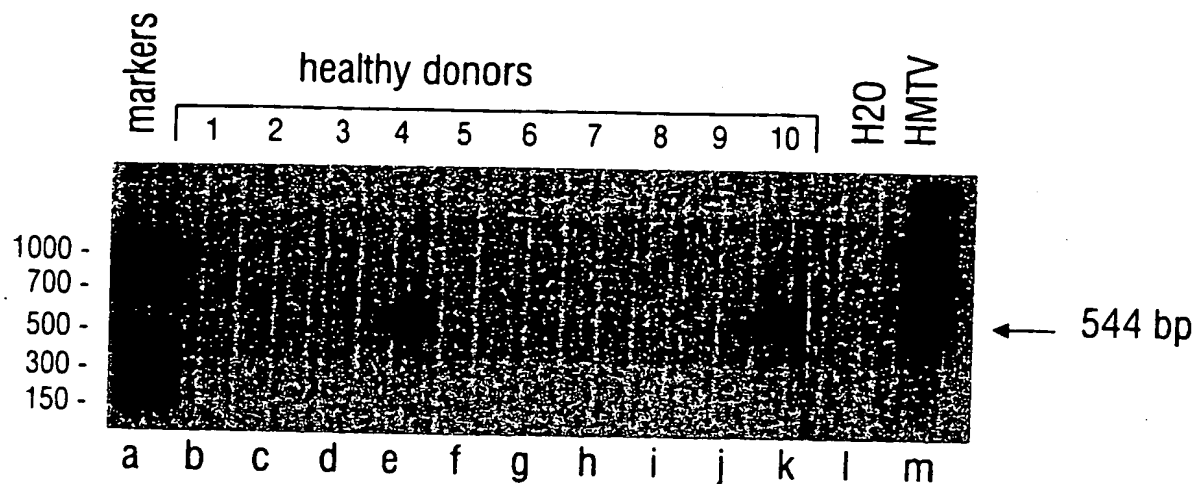


FIG. 2A

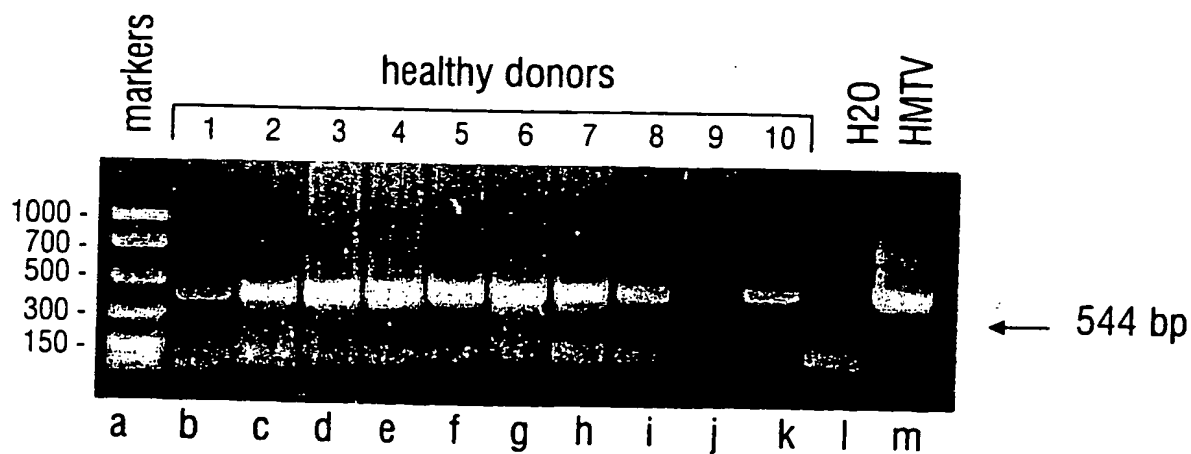


FIG. 2B

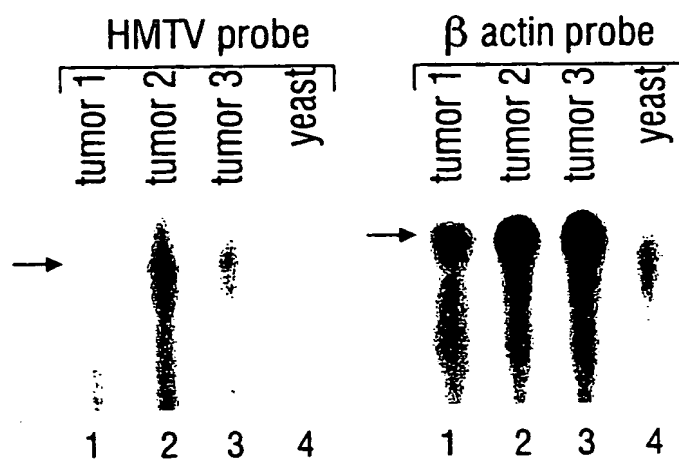


FIG. 3



- 1 -

## SEQUENCE LISTING

&lt;110&gt; Garry, Robert F.

5 &lt;120&gt; Human Endogenous Retrovirus in Breast Cancer

&lt;130&gt; TUMC:012

&lt;140&gt;

10 &lt;141&gt;

&lt;160&gt; 30

&lt;170&gt; PatentIn Ver. 2.1

15

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&lt;211&gt; 461

&lt;212&gt; DNA

&lt;213&gt; Mouse mammary tumor virus

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 ctagttcccc atacagaatt gtttcgctta gttgcagcct caagatatct tattctcaaa 240  
 aggccaggat ttcaagaaca tgacatgatt cctacatctg cctgtgttac ttacccttat 300  
 gccatattat taggattacc tcagctaata gatatagaga aaagaggatc tacttttcat 360  
 atttcctggt cttctttag attgactaat tgtttagatt cttctgccta cgactatgca 420  
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&lt;210&gt; 2

&lt;211&gt; 461

&lt;212&gt; DNA

35 &lt;213&gt; Human Mammary Tumor Virus

&lt;400&gt; 2

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 gaacctacat gggtctggga aaattctcct aaggatccca atgatagaga ttttactgct 180  
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 gccatattat taggattacc tcagctaata gatatagaaa aaagaggatc tacttttcat 360  
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&lt;210&gt; 3

&lt;211&gt; 461

50 &lt;212&gt; DNA

&lt;213&gt; Human Mammary Tumor Virus

&lt;400&gt; 3

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 gaacctactt gggtctggga aaattctcct aaggatccca atgatagaga ttttactgct 180  
 ctagttcccc atacagaatt gtttcgctta gttgcagcct caagacatct tattctcaaa 240

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aagccaggat ttcaagaaca tgagatgatt cctacatctg cctgtgttac ttacccttat 300
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atttcctgtt cttcttgttg attgactaat tgttttagatt cttctgccta cgactatgca 420
gcgatcatag tcaagaggcc gccatacgtg ctgctacctg t 461

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&lt;210&gt; 4

&lt;211&gt; 461

&lt;212&gt; DNA

10 &lt;213&gt; Human Mammary Tumor Virus

&lt;400&gt; 4

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atttcctgtt cttcttgtag attgactaat tgttttagact cttctgccta cgactatgca 420
20 gcgatcatag tcaagaggcc gccatacgtg ctgctacctg t 461

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&lt;211&gt; 461

25 &lt;212&gt; DNA

&lt;213&gt; Rhesus Mammary Tumor Virus

&lt;400&gt; 5

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gaacctactt gggtctggga aaattctcct aaagatccca atgatagaga ttttactgct 180
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35 atttcctgtt cttcttgtag attgactaat tgttttagatt cttctgccta cgactatgca 420
gcgatcatag tcaagaggcc gccatacgtg ctgctacctg t 461

```

&lt;210&gt; 6

40 &lt;211&gt; 461

&lt;212&gt; DNA

&lt;213&gt; Rhesus Mammary Tumor Virus

&lt;400&gt; 6

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45 ggaaattata ctccccctgt caataaagag gttcatcgat ggtatgaagc aggatgggta 120
gaacctactt gggtctggga aaattctcct aaagatccca atgatagaga ttttactgct 180
ctagttcccc atacagaatt gtttcgctta gttgcagcct caagacatct tattctcaaa 240
aagccaggat ttcaagaaca tgagatgatt cctacatctg cctgtgttac ttacccttat 300
50 gccatattat taggattacc tcagctaata gatatagaga aaagaggatc tacttttcat 360
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```

55 &lt;210&gt; 7

&lt;211&gt; 461

&lt;212&gt; DNA

- 3 -

&lt;213&gt; Cat Mammary Tumor Virus

&lt;400&gt; 7.

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   ggaaattata ctccccctgt caataaagag gtccatcgat ggtatgaagc aggatgggta 120
   gaacctacat gggtctggga aaattctcct aaggatccca atgatagaga ttttattgct 180
   ctagttcccc atacagaatt gtttcgctta gttgcagcct caagacatct tattctcaaa 240
   aagccaggat ttcaagaaca taagatgatt cctacatctg cctgtgttac ttacccttat 300
   gccatattat taggattacc tcagctaata gatatagaga aaagaggatc tacttttcat 360
10 atttcctgtt cttctttag attgactaat tgtttagatt cttctgccta cgactatgca 420
   gcgatcatag tcaagaggcc gccatacgtg ctgctacctg t
                                     461

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&lt;210&gt; 8

15 &lt;211&gt; 461

&lt;212&gt; DNA

&lt;213&gt; Cat Mammary Tumor Virus

&lt;400&gt; 8

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   ctagttcccc atacagaatt gtttcgctta gttgcagcct caagacatct tattctcaaa 240
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   atttcctgtt cttctttag attgactaat tgtttagatt cttctgccta cgactatgca 420
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                                     461

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30 &lt;210&gt; 9

&lt;211&gt; 104

&lt;212&gt; DNA

&lt;213&gt; Mouse mammary tumor virus

35 &lt;400&gt; 9

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   aattcattgt gtcaggatct catccgcccc atacgaaaaa cagg
                                     104

```

40 &lt;210&gt; 10

&lt;211&gt; 104

&lt;212&gt; DNA

&lt;213&gt; Cat Mammary Tumor Virus

45 &lt;400&gt; 10

```

   atgatgccga gaggagaagg gtcagatata ttgatcaaac aattggcgta aaaaaatgca 60
   aattcattgt gccaatgctt tatccgtcca atacgaaaaa cagg
                                     104

```

50 &lt;210&gt; 11

&lt;211&gt; 153

&lt;212&gt; PRT

&lt;213&gt; Mouse mammary tumor virus

55 &lt;400&gt; 11

```

   Ser Leu Pro Ser Pro Ser Val Asp Gln Ser Asp Gln Ile Lys Ser Lys
     1               5               10               15

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- 4 -

Lys Asp Leu Phe Gly Asn Tyr Thr Pro Pro Val Asn Lys Glu Val His  
                     20                    25                    30

5 Arg Trp Tyr Glu Ala Gly Trp Val Glu Pro Thr Trp Phe Trp Glu Asn  
                     35                    40                    45

Ser Pro Lys Asp Pro Asn Asp Arg Asp Phe Thr Ala Leu Val Pro His  
                     50                    55                    60

10 Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg Tyr Leu Ile Leu Lys  
                     65                    70                    75                    80

15 Arg Pro Gly Phe Gln Glu His Asp Met Ile Pro Thr Ser Ala Cys Val  
                     85                    90                    95

Thr Tyr Pro Tyr Ala Ile Leu Leu Gly Leu Pro Gln Leu Ile Asp Ile  
                     100                    105                    110

20 Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Arg Leu  
                     115                    120                    125

Thr Asn Cys Leu Asp Ser Ser Ala Tyr Asp Tyr Ala Ala Ile Ile Val  
                     130                    135                    140

25 Lys Arg Pro Pro Tyr Val Leu Leu Pro  
                     145                    150

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     <212> PRT  
     <213> Human Mammary Tumor Virus

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                     20                    25                    30

Arg Trp Tyr Glu Ala Gly Trp Val Glu Pro Thr Trp Phe Trp Glu Asn  
                     35                    40                    45

45 Ser Pro Lys Asp Pro Asn Asp Arg Asp Phe Thr Ala Leu Val Pro His  
                     50                    55                    60

Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg Tyr Leu Ile Leu Lys  
                     65                    70                    75                    80

50 Arg Pro Gly Phe Gln Glu His Asp Met Ile Pro Thr Ser Ala Cys Val  
                     85                    90                    95

55 Thr Tyr Pro Tyr Ala Ile Leu Leu Gly Leu Pro Gln Leu Ile Asp Ile  
                     100                    105                    110

Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Arg Leu

- 5 -

115                      120                      125  
 Thr Asn Cys Leu Asp Ser Ser Ala Tyr Asp Tyr Ala Ala Ile Ile Val  
     130                      135                      140  
 5 Lys Lys Ala Pro Tyr Val Leu Leu Pro  
     145                      150  
  
 10 <210> 13  
     <211> 152  
     <212> PRT  
     <213> Human Mammary Tumor Virus  
  
 15 <400> 13  
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         1                      5                      10                      15  
     Lys Asp Leu Phe Gly Asn Tyr Thr Pro Pro Val Asn Lys Glu Val His  
 20                      20                      25                      30  
     Arg Trp Tyr Glu Ala Gly Trp Val Glu Pro Thr Trp Phe Trp Glu Asn  
         35                      40                      45  
 25 Ser Pro Lys Asp Pro Asn Asp Arg Asp Phe Thr Ala Leu Val Pro His  
         50                      55                      60  
     Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg His Leu Ile Leu Lys  
 30                      65                      70                      75                      80  
     Lys Pro Gly Phe Gln Glu His Glu Met Ile Pro Thr Ser Ala Cys Val  
                             85                      90                      95  
 35 Thr Tyr Pro Tyr Ala Ile Leu Leu Gly Leu Pro Gln Leu Ile Asp Ile  
         100                      105                      110  
     Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Leu Thr  
         115                      120                      125  
 40 Asn Cys Leu Asp Ser Ser Ala Tyr Asp Tyr Ala Ala Ile Ile Val Lys  
         130                      135                      140  
     Arg Pro Pro Tyr Val Leu Leu Pro  
 45                      145                      150  
  
     <210> 14  
     <211> 153  
     <212> PRT  
 50 <213> Human Mammary Tumor Virus  
  
     <400> 14  
     Ser Leu Pro Ser Pro Ser Ile Glu Gln Ser Asn Gln Ile Lys Ser Lys  
         1                      5                      10                      15  
 55 Lys Asp Leu Leu Gly Asn Tyr Thr Pro Pro Val Asn Lys Glu Val His  
         20                      25                      30

- 6 -

Arg Trp Tyr Glu Ala Gly Trp Val Glu Pro Thr Trp Phe Trp Glu Asn  
                   35                                  40                                  45

5 Ser Pro Lys Asp Pro Asn Asp Arg Asp Phe Thr Ala Leu Val Pro His  
           50                                  55                                  60

Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg His Leu Ile Leu Lys  
   65                                  70                                  75                                  80

10 Arg Pro Gly Phe Gln Glu His Glu Met Ile Pro Thr Ser Ala Cys Val  
                                   85                                  90                                  95

Thr Tyr Pro Tyr Ala Ile Leu Leu Gly Leu Pro Gln Leu Ile Asp Ile  
 15                                  100                                  105                                  110

Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Arg Leu  
           115                                  120                                  125

20 Thr Asn Cys Leu Asp Ser Ser Ala Tyr Asp Tyr Ala Ala Ile Ile Val  
           130                                  135                                  140

Lys Arg Pro Pro Tyr Val Leu Leu Pro  
 25          145                                  150

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 30 <213> Rhesus Mammary Tumor Virus

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   1                                  5                                  10                                  15

35 Lys Asp Leu Phe Gly Asn Tyr Thr Pro Pro Val Asn Lys Glu Val His  
                                   20                                  25                                  30

Arg Trp Tyr Glu Ala Gly Trp Val Glu Pro Thr Trp Phe Trp Glu Asn  
 40                  35                                  40                                  45

Ser Pro Lys Asp Pro Asn Asp Arg Asp Phe Thr Ala Leu Val Pro His  
           50                                  55                                  60

45 Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg His Leu Ile Leu Lys  
           65                                  70                                  75                                  80

Lys Pro Gly Phe Gln Glu His Glu Met Ile Pro Thr Ser Ala Cys Val  
                                   85                                  90                                  95

50 Thr Tyr Pro Tyr Ala Ile Leu Leu Gly Leu Pro Gln Leu Ile Asp Ile  
                                   100                                  105                                  110

Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Arg Leu  
 55          115                                  120                                  125

Thr Asn Cys Leu Asp Ser Ser Ala Tyr Asp Tyr Ala Ala Ile Ile Val

- 7 -

130                      135                      140  
 Lys Arg Pro Pro Tyr Val Leu Leu Pro  
 145                      150  
 5  
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 15 Lys Asp Leu Phe Gly Asn Tyr Thr Pro Pro Val Asn Lys Glu Val His  
                     20                      25                      30  
 20 Arg Trp Tyr Glu Ala Gly Trp Val Glu Pro Thr Trp Phe Trp Glu Asn  
                     35                      40                      45  
 Ser Pro Lys Asp Pro Asn Asp Arg Asp Phe Thr Ala Leu Val Pro His  
 50                      55                      60  
 25 Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg His Leu Ile Leu Lys  
 65                      70                      75                      80  
 Lys Pro Gly Phe Gln Glu His Glu Met Ile Pro Thr Ser Ala Cys Val  
                     85                      90                      95  
 30 Thr Tyr Pro Tyr Ala Ile Leu Leu Gly Leu Pro Gln Leu Ile Asp Ile  
                     100                      105                      110  
 35 Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Arg Pro  
                     115                      120                      125  
 Thr Asn Cys Leu Asp Ser Ser Ala Tyr Asp Tyr Ala Ala Ile Ile Val  
 130                      135                      140  
 40 Lys Arg Pro Pro Tyr Val Leu Leu Pro  
 145                      150  
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 45 <211> 153  
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 Lys Asp Leu Leu Gly Asn Tyr Thr Pro Pro Val Asn Lys Glu Val His  
                     20                      25                      30  
 55 Arg Trp Tyr Glu Ala Gly Trp Val Glu Pro Thr Trp Phe Trp Glu Asn  
                     35                      40                      45

- 8 -

Ser Pro Lys Asp Pro Asn Asp Arg Asp Phe Thr Ala Leu Val Pro His  
 50 55 60

5 Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg His Leu Ile Leu Lys  
 65 70 75 80

Lys Pro Gly Phe Gln Glu His Lys Met Ile Pro Thr Ser Ala Cys Val  
 85 90 95

10 Thr Tyr Pro Tyr Ala Ile Leu Leu Arg Leu Pro Gln Leu Ile Asp Ile  
 100 105 110

Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Arg Leu  
 115 120 125

15 Thr Asn Cys Leu Asp Ser Ser Ala Tyr Asp Tyr Ala Ala Ile Ile Val  
 130 135 140

20 Lys Arg Pro Pro Tyr Val Leu Leu Pro  
 145 150

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 20 25 30

35 Arg Trp Tyr Glu Ala Gly Trp Val Glu Pro Thr Trp Phe Trp Glu Asn  
 35 40 45

Ser Pro Lys Asp Pro Asn Asp Arg Asp Phe Thr Ala Leu Val Pro His  
 40 50 55 60

Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg His Leu Ile Leu Lys  
 65 70 75 80

45 Lys Pro Gly Phe Gln Glu His Lys Met Ile Pro Thr Ser Ala Cys Val  
 85 90 95

Thr Tyr Pro Tyr Ala Ile Leu Leu Arg Leu Pro Gln Leu Ile Asp Ile  
 100 105 110

50 Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Arg Leu  
 115 120 125

Thr Asn Cys Leu Asp Ser Ser Ala Tyr Asp Tyr Ala Ala Ile Ile Val  
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Lys Arg Pro Pro Tyr Val Leu Leu Pro



- 9 -

145

150

&lt;210&gt; 19

5 &lt;211&gt; 35

&lt;212&gt; PRT

&lt;213&gt; Mouse mammary tumor virus

&lt;400&gt; 19

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 1 5 10 15

Trp Glu Lys Cys Lys Phe Ile Val Ser Gly Ser His Pro Pro Asn Thr  
 20 25 30

15

Lys Asn Arg  
 35

20 &lt;210&gt; 20

&lt;211&gt; 35

&lt;212&gt; PRT

&lt;213&gt; Cat Mammary Tumor Virus

25 &lt;400&gt; 20

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 1 5 10 15

30 Tyr Lys Lys Cys Lys Phe Ile Val Pro Arg Ser Tyr Pro Ser Asn Thr  
 20 25 30

Lys Asn Arg  
 35

35

&lt;210&gt; 21

&lt;211&gt; 460

&lt;212&gt; DNA

&lt;213&gt; Human Mammary Tumor Virus

40

&lt;400&gt; 21

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 aacctacatg gttctgggaa aattctccta aggatcccaa tgatagagat ttactgctc 180  
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 tttcctgttc ttcttgtaaa ttgactaatt gtttagattc ttctgcctac gactatgcag 420  
 cgatcatagt caagaaggcg ccatatgtgc tgctacctgt 460

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&lt;210&gt; 22

&lt;211&gt; 38

&lt;212&gt; PRT

55 &lt;213&gt; Human Mammary Tumor Virus

&lt;400&gt; 22

- 10 -

Ser Leu Pro Ser Pro Ser Val Asp Gln Ser Asp Gln Ile Lys Ser Lys  
 1 5 10 15

Arg Ile Tyr Leu Glu Ile Ile Leu Pro Leu Ser Ile Lys Phe Ile Gly  
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Gly Met Lys Gln Asp Gly  
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10

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 gccatattat taggattacc tcagctaata gatatagaaa aaagaggatc tacttttcat 360  
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35

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Arg Trp Tyr Glu Ala Gly Trp Val Glu Pro Thr Trp Phe Trp Glu Asn  
 35 40 45

40

Ser Pro Lys Asp Pro Asn Asp Arg Asp Phe Thr Ala Leu Val Pro His  
 50 55 60

45

Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg Tyr Leu Ile Leu Lys  
 65 70 75 80

Arg Pro Gly Phe Gln Glu His Asp Met Ile Pro Thr Ser Ala Cys Val  
 85 90 95

50

Thr Tyr Pro Tyr Ala Ile Leu Leu Gly Leu Pro Gln Leu Ile Asp Ile  
 100 105 110

Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Arg Leu  
 115 120 125

55

Thr Asn Cys Leu Asp Ser Ser Ala Tyr Asp Tyr Ala Ala Ile Ile Val

- 11 -

130

135

140

Lys Lys Ala Pro Tyr Val Leu Leu Pro  
 145 150

&lt;210&gt; 25

&lt;211&gt; 461

&lt;212&gt; DNA

10 <213> Human Mammary Tumor Virus

&lt;400&gt; 25

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 gaacctacat ggttctggga aaattctcct aaggatccca atgatagaga ttttactgct 180  
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 gccatattat taggattacc tcagctaata gatatagaaa aaagaggatc tacttttcat 360  
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 20 gcgatcatag tcaagaaggc gccatattgt ctgctacctg t 461

&lt;210&gt; 26

&lt;211&gt; 153

&lt;212&gt; PRT

&lt;213&gt; Human Mammary Tumor Virus

&lt;400&gt; 26

30 Ser Leu Pro Ser Pro Ser Val Asp Leu Ser Asp Gln Ile Lys Ser Lys  
 1 5 10 15  
 Lys Asp Leu Phe Gly Asn Tyr Thr Pro Pro Val Asn Lys Gly Val His  
 20 25 30  
 35 Arg Trp Tyr Glu Ala Gly Trp Val Glu Pro Thr Trp Phe Trp Glu Asn  
 35 40 45  
 Ser Pro Lys Asp Pro Asn Asp Arg Asp Phe Thr Ala Leu Val Pro His  
 50 55 60  
 40 Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg Tyr Leu Ile Leu Lys  
 65 70 75 80  
 Arg Pro Gly Phe Gln Glu His Asp Met Ile Pro Thr Ser Ala Cys Val  
 45 85 90 95  
 Thr Tyr Pro Tyr Ala Ile Leu Leu Gly Leu Pro Gln Leu Ile Asp Ile  
 100 105 110  
 50 Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Arg Leu  
 115 120 125  
 Thr Asn Cys Leu Asp Ser Ser Ala Tyr Asp Tyr Ala Ala Ile Ile Val  
 130 135 140  
 55 Lys Lys Ala Pro Tyr Val Leu Leu Pro  
 145 150

- 12 -

&lt;210&gt; 27

&lt;211&gt; 460

5 &lt;212&gt; DNA

&lt;213&gt; Human Mammary Tumor Virus

&lt;400&gt; 27

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tcccttccct cgcctagtgt agatcagtcg gatcagatta aaagcaaaaa ggatctattt 60
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&lt;210&gt; 28

20 &lt;211&gt; 152

&lt;212&gt; PRT

&lt;213&gt; Human Mammary Tumor Virus

&lt;400&gt; 28

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25 Ser Leu Pro Ser Pro Ser Val Asp Leu Ser Asp Gln Ile Lys Ser Lys
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Lys Asp Leu Phe Gly Asn Tyr Thr Pro Pro Val Asn Lys Glu Val His
    20             25             30

30 Arg Trp Tyr Glu Ala Gly Trp Val Glu Pro Thr Trp Phe Trp Glu Asn
    35             40             45

Ser Pro Lys Asp Pro Asn Asp Arg Asp Phe Thr Ala Leu Val Pro His
    50             55             60

Thr Glu Leu Phe Arg Leu Val Ala Ala Ser Arg Tyr Leu Ile Leu Lys
    65             70             75             80

40 Arg Pro Gly Phe Gln Glu His Asp Met Ile Pro Thr Ser Ala Cys Val
    85             90             95

Thr Tyr Pro Tyr Ala Ile Leu Leu Gly Leu Pro Gln Leu Ile Asp Ile
    100            105            110

45 Glu Lys Arg Gly Ser Thr Phe His Ile Ser Cys Ser Ser Cys Arg Leu
    115            120            125

Thr Asn Cys Leu Asp Ser Ser Ala Tyr Glu Tyr Ala Ala Ile Ile Val
    130            135            140

Lys Lys Gly His Met Cys Cys Leu
    145            150

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55

&lt;210&gt; 29

&lt;211&gt; 461

- 13 -

&lt;212&gt; DNA

&lt;213&gt; Human Mammary Tumor Virus

&lt;400&gt; 29

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5  tcccttccct cgcctagtgt agatcagtca gatcagatta aaaacaaaaa ggatctattt 60
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   ctagttcccc atacagaatt gttccgctta gttgcagcct caagacatct tattctcaaa 240
   aagccaggat ttcaagaaga tgacatgatt cctacatctg cctgtgttac ttacccttat 300
10 gccatattat taggattacc tcagctaata gatatagaga aaagaggatc tacttttcat 360
   atttcctgtt cttctttag attgactaat tgtttagatt cttctgccta cgactatgca 420
   gcgatcatag tcaagaaggc gccatacgtg ctgctacctg t
                                     461

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15 &lt;210&gt; 30

&lt;211&gt; 43

&lt;212&gt; PRT

&lt;213&gt; Human Mammary Tumor Virus

20 &lt;400&gt; 30

```

Ser Leu Pro Ser Pro Ser Val Asp Gln Ser Asp Gln Ile Lys Asn Lys
  1             5             10             15

```

```

25  Lys Asp Leu Phe Gly Asn Tyr Thr Pro Pro Val Asn Lys Glu Val His
      20             25             30

```

```

Arg Trp Tyr Glu Ala Gly Trp Val Glu Pro Thr
  35             40

```

30

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 00/18279

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 C12N15/12 C07K14/47 C07K16/18 C12Q1/68 G01N33/566  
//C12N15/48,C07K14/15,C07K16/08

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, EMBL, BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 17470 A (HOLLAND JAMES F) 15 May 1997 (1997-05-15) the whole document ---	1-26
X	WANG Y. ET AL.: "Detection of mammary tuor virus env gene-like sequences in human breast cancer." CANCER RES., vol. 55, 15 November 1995 (1995-11-15), pages 5173-5179, XP002150741 cited in the application the whole document --- -/-	1-26

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*G\* document member of the same patent family

Date of the actual completion of the international search

12 January 2001

Date of mailing of the international search report

30. 01. 2001

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Galli, I

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 00/18279

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WANG Y. ET AL.: "Expression of mouse mammary tumor virus-like env gene sequences in human breast cancer." CLIN. CANCER RES., vol. 4, no. 10, October 1998 (1998-10), pages 2565-2568, XP000953033 the whole document	1-26
X	REDMOND S.M.S. & DICKSON C.: "Sequence and expression of the mouse mammary tumor virus env gene." EMBO J., vol. 2, 1983, pages 125-131, XP000971882 compare Fig 2 with seq. 5,6,7,8,10.	1-26
A	FAFF O. ET AL.: "Retrovirus-like particles from the human T47D cell line are related to mouse mammary tumor virus and are of human endogenous origin." J. GEN. VIROL., vol. 73, 1992, pages 1087-1097, XP002157129 the whole document	1-26

# INTERNATIONAL SEARCH REPORT

national application No.  
PCT/US 00/18279

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.



FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-26, partly

An isolated molecule comprising a DNA sequences that has at least 99% identity with seq. IDs 2,3,4,21,23,25,27 or 29 or fragments thereof.

Corresponding compositions, polypeptides, antibodies, RNAs, vectors, methods of detection, diagnostic kits.

2. Claims: 1-26, partly

Idem as subject-matter 1 but limited to sequence IDs 5,6.

3. Claims: 1-26, partly

Idem as subject-matter 1 but limited to sequence IDs 7,8,10.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/18279

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9717470 A	15-05-1997	US 5686247 A	11-11-1997
		AU 717291 B	23-03-2000
		AU 1271497 A	29-05-1997
		EP 0866878 A	30-09-1998
		JP 2000500650 T	25-01-2000
		US 6040146 A	21-03-2000

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